Assessing the Impacts of Habitat Fragmentation and Subsequent Anthropogenic Expansion on the Behavioural, Nesting and Population Ecology of the Estuarine Crocodile, *Crocodylus porosus*.

By

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Summary

The project sought to examine the effects of habitat loss and fragmentation on the ecology and population genetics of the estuarine crocodile (*Crocodylus porosus*). Additionally, the role played by humans in this anthropogenically-altered landscape was examined. Through the utilisation of a host of technologies, some previously established, some completely novel in crocodilian research, a new insight into how the landscape is utilised by these cryptic predators was developed. This project represents a first detailed look at Sabah's crocodilian population, as well as being the first active crocodile research carried out in Sabah's longest river.

Male crocodiles were found to adhere to one of two behavioural strategies, territorial and nomadic, mirroring findings of Campbell et al. (2013). Territory sizes were, however, found to be smaller than those described in Australia, this was attributed to increased prey availability and ecosystem productivity. Only two females were tagged and appeared to also display differences in behavioural strategy. However, due to the small sample size, further work is required to confirm this. Both males and females were found to avoid barriers and were unwilling to pass beyond the barrier, despite no physical obstruction. Nests were detectable aerially through the use of drones and medium-large scale surveys shown to be feasible. Nests were found to all display a number of similarities in terms of habitat characteristics, allowing for refined modelling of survey locations. This allows for a larger survey area to be completed given a limited number of flights, highlighting its cost effectiveness versus traditional methods of nest surveying. Genetic analysis suggested that there was no evidence of a genetic bottleneck following the population recovery that has occurred over the last 30 years. Geographically indistinct haplogroups were discovered, as well as limited levels of inbreeding. The project also indicated that the population studied had undergone a population expansion that seems to have coincided with the onset of the last ice age and is likely attributable to changes in climate.

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Chapter 1 – General Introduction

1.1 Regional Biogeography

Tropical rainforests represent expansive, understudied ecosystems, with those of Sundaland, comprising the islands of Borneo, Sumatra and Java, as well as peninsula Malaysia, being among the most biologically diverse (Myers et al. 2000). Borneo is the third largest island on Earth, covering an area of some 743,000 km^2; it is bisected by the Equator and typifies many people's definitions of equatorial tropical rainforest. Its forests are estimated to be among the oldest on Earth (Brühl et al. 2003), dating back some 140 million years. Large-scale land conversion of the region has, however, resulted in a projected loss of three quarters of Southeast Asian biodiversity by the year 2100 (Sala et al. 2000; Sodhi et al. 2004). Despite this loss, the area has retained a high proportion of its endemic biodiversity, with some 25,000 plant species found throughout Sundaland (Myers et al. 2000). Contemporary land-use conversion in the region has been focussed almost solely on large-scale cultivation of oil palm (*Elaeis guineensis*), with Sundaland being one of the most heavily converted regions (Koh & Wilcove 2007). This trend has been cited as a major factor in a projected future biodiversity collapse in the region (Fitzherbert et al. 2008; Sodhi et al. 2004).

Sabah is one of two Malaysian states that comprise much of North Borneo. It formed part of the medieval Bruneian Empire and later the Sulu Sultanate. Between 1761 and 1963 it formed part of British North Borneo, gaining independence and subsequently joining Malaysia in 1966. The state of Sabah has itself become a global hub for oil palm production with, as of 2011, an estimated 19.3% of the state's total land area being devoted to oil palm agriculture (Abram et al. 2014). This has led to many of the state's forests becoming fragmented (Fig.1.1).

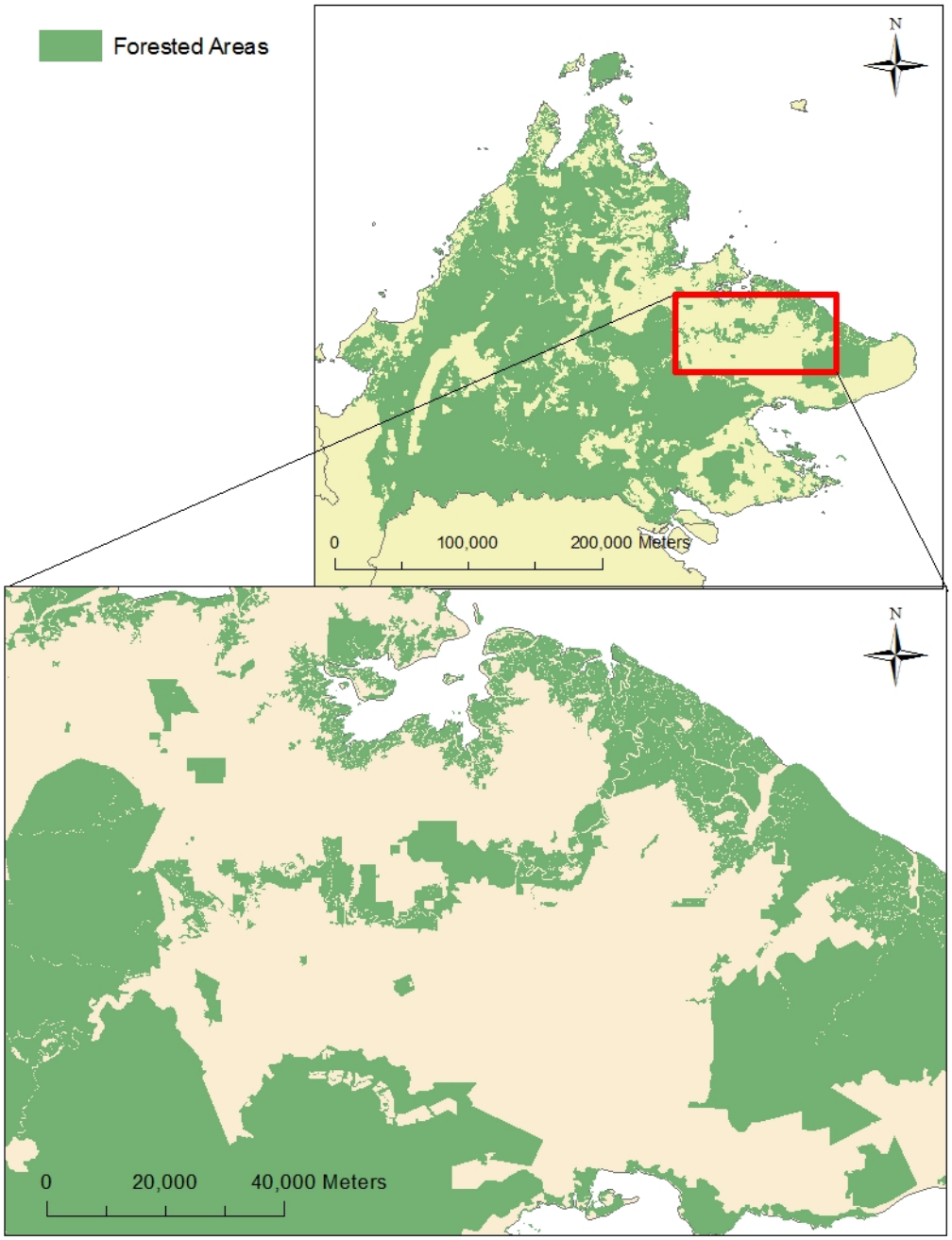

Figure 1.1 Current extent of forest cover in Sabah (inset) and the lower Kinabatangan floodplain. The Kinabatangan region is clearly shown as one of the most heavily fragmented regions in Sabah.

1.2 Lower Kinabatangan Wildlife Sanctuary

The Lower Kinabatangan Wildlife Sanctuary (LKWS) was first gazetted in 2005 and comprises 10 forested "lots" (patches of protected forest), totalling an area of some 27,000 ha (Goossens et al. 2005). These lots retain varying levels of connectivity with some completely encompassed by active agriculture. The

LKWS is situated in the expansive floodplain of the Kinabatangan River, an area with a catchment of around 520,000 ha (Abram et al. 2014). At a length of 560 km, the river is the second longest in Borneo, and the longest in Sabah (Boonratana 2000). The remaining forest is not only highly fragmented (Figure 1.1) but also highly logged and regenerating in terms of forest regrowth (Goossens et al. 2005). Despite the relatively small patches of remaining habitat, a large proportion of the original biodiversity remains, with records of 10 primate species (Lackman-Ancrenaz et al. 2001), 314 avian species and 101 species of reptile (Lackman-Ancrenaz & Manokaran 2008).

As with many other areas in Sabah, the Kinabatangan River has seen an expansion of anthropogenic presence over recent decades (Primack & Hall 1992). A consequence of this has been the appearance of an ever-increasing number of villages along the riverbank, with an associated increase in fishing and hunting pressures. This is despite the illegality of hunting within a state-gazetted wildlife sanctuary. In addition, the past 20 years has seen further anthropogenic expansions, including that of several important socio-economic villages throughout the river, fuelling the burgeoning ecotourism sector (Hussin et al. 2008). These increases in human presence have inevitable consequences for rates of wildlife conflict, especially in circumstances where habitat has become increasingly limited.

1.3 Human-wildlife conflict in Sabah

Although they are found throughout much of the Sundaland, large terrestrial carnivores such as tigers (*Panthera tigris*) and leopards (*Panthera pardus*) are absent from the island of Borneo. Borneo's only extant member of the large felid group is the Sunda clouded leopard (*Neofelis diardi*); this species is small and poses little threat to humans or their interests. The majority of human-wildlife conflict in Sabah stems from the Bornean elephant (*Elephas maximus borneensis*) and the estuarine crocodile (*Crocodylus porosus*).

Bornean elephants within LKWS number approximately 200 individuals (English et al. 2015); they are responsible for many acts of property destruction

and the occasional confrontation with humans. This is mostly due to habitat-based constraints, which are particularly prevalent in the LKWS. Estuarine crocodiles are less associated with property destruction, although livestock and pets are sometimes at risk, but are more associated with attacks and aggression towards humans. Official figures cite the number of deaths in Sabah between 2000 and 2011, arising from crocodile interactions as 15 (Sabah Wildlife Department *unpubl. data*). This is likely to be an underestimation arising from under-reporting, especially from within plantations.

Estuarine crocodiles were heavily hunted in the 20th Century and, as a result, experienced a substantial reduction in population numbers throughout their habitat range (Thorbjarnarson et al. 1998). Their subsequent recovery (Fukuda et al. 2011) has left unanswered questions regarding the genetic health of populations. Apex predators, of which the estuarine crocodile is an obvious example, are often those most greatly affected by habitat destruction; this is because they often require far more habitat area per individual than many of their prey species (Crooks et al. 2011). Crocodilians, with their ability to traverse waterways and travel long distances, are not confined by habitat fragmentation to the same extent as many terrestrial predators (Kay 2004A; Read et al. 2007), however, they do require large prey bases and increased habitat patchiness can lead to serious declines in prey availability through a reduction in biodiversity (Fitzherbert et al. 2008). A consequence of this reduction can be an increase in the levels of human-crocodile conflict, either due to territorial defence, or active hunting of humans by crocodilians. A less abundant prey base could also have implications for how crocodilians behave in fragmented landscapes. The shared resources competed for as prey items, such as fish, by crocodilians and humans is a major driver for increased conflict. Increasing our understanding of these behaviours have potentially important management and safety implications for the local ecosystem, and also have wider applicability to a host of ecosystems across the world, especially in areas where rainforest ecosystems are increasingly under threat of habitat fragmentation.

1.4 Thesis Aims

The overall aim of this study was to examine how the estuarine crocodile is affected by fragmented habitat; this was attempted by investigating its spatial ecology, nesting distributions and population genetics. These different aspects were explored using a range of field- and laboratory-based technologies and approaches that included: genetic analyses, the use of existing and novel forms of satellite tracking technologies, and the application, for the first time in crocodilian research, of an Unmanned Aerial Vehicle (UAV), generally referred to as a "drone".

Chapter 2 provides a review of the literature relevant to the current study; crocodilian biology, nesting ecology, the burgeoning role of drones as a conservation tool, and the history of genetic analysis and its uses in crocodilian research are all explored. Through this literature review it is aimed to provide relevant background to ensure a contextual setting for each of the following empirical chapters.

Chapter 3 investigates the home-ranging and spatial ecology of *C. porosus*. Three different satellite tracking technologies were employed to determine the optimum method of tracking individuals in equatorial rainforest ecosystems. Continual tracking techniques were also employed, not only to establish home range estimates, but to look at activity patterns and fine-scale spatial distribution, as well as to understand how individuals are utilising the landscape better.

Chapter 4 provides a verification of the applicability of one methodological approach to crocodile nest identification. A novel technique, this "proof of concept" sought to provide a cost-effective and repeatable means of surveying large areas of nesting habitat, whilst ensuring the safety of field personnel. A version of this study has been published in *Herpetological Conservation and Biology*.

Chapter 5 seeks to build on the aerial nest detection methodologies described in Chapter 4, employing them to examine the spatial nesting ecology of crocodilian nesting in a fragmented rainforest habitat. The main aim of this specific investigation was to refine the identification of factors key to nest detection and subsequently model the most likely nest-harbouring habitat areas. The possibilities of using these results in refining aerial mission planning and in the reduced effort of equivalent nest detection rates are discussed.

In Chapter 6 the population genetics of the Kinabatangan River's crocodiles are explored, with an attempt to place them within the genetic context of the species. Through the use of mitochondrial and microsatellite analysis, demographic history, population structure and their effects on the crocodile population are explored.

Finally, in Chapter 7, by summarising and integrating the information from each chapter, an attempt is made to give an overview of crocodilian ecology and population genetics in a fragmented landscape. The study is also placed in the context of management, conservation and conflict implications of the findings in terms of the crocodile populations of the Kinabatangan. This project will not only have applicability in Sabah but throughout the region and potentially worldwide. Information gaps and areas demanding further research attention are also highlighted.

Chapter 2 - Literature Review

2.1 Crocodilians

The state of Sabah is home to a thriving population of estuarine crocodiles (*Crocodylus porosus*); there are also isolated reports of a second species, the tomistoma (*Tomistoma schlegelii*). With the number of the latter species declining across their range in recent decades, the conservation plight of tomistomas has been brought into sharp focus among crocodilian researchers; this resulted in the formation of the Tomistoma Task Force (Tomistoma.org) that has conducted survey work to obtain population estimates. Whilst Sabah has no confirmed recorded sightings of tomistoma, there are reportedly unconfirmed sightings from the Klias and Padas Rivers, located in Western Sabah (Whitaker 1984). If a tomistoma population does exist within Sabah it would be most likely found closest to other extant populations, the largest of which can be found in Sarawak (Stuebing et al. 2006).

Crocodilians represent part of an ancient clade of species, known as the Diapsids, within Class Reptilia (Rieppel & deBraga 1996). The group is distinguishable from other reptilians in their possession of two holes located behind the eyes, known as temporal fenestrae (Brochu 2006). Crocodilians, along with extinct 'crocodile-like reptile' species (Pseudosuchia), several species of modern day bird (Avemetatarsalia – 'bird-like reptiles'), as well as many extinct and extant species of early birds (Toljagic & Butler 2013), form part of the sub-clade Archasaurs. The evolution of modern crocodilians can be traced back to the Late Triassic, 210-230 million years ago (Bakker 1971). Following the mass extinction event that occurred at the Triassic-Jurassic boundary, early crocodilians, or crocodylomorphs, began to radiate evolutionarily, filling niches vacated by organisms that had become extinct (Toljagic & Butler 2013). During the Early Jurassic, a range of insectivorous, piscatory and herbivorous crocodylomorphs evolved, joining the carnivorous species that had evolved during the Late Triassic (Stubbs et al. 2013; Toljagic & Butler 2013). Recognisable species of crocodilian began to appear by the Campanian (approximately 80 million years ago), with both alligator and garial

lineages present by the Maastrichtian (approximately 70 million years ago) (Brochu 2003).

There are 24 extant crocodilian species; this number has recently been elevated, from 23, as a result of genetic findings carried out on a sub-population of Nile crocodiles (*Crocodylus niloticus*) (Hekkala et al. 2011). First discovered by Saint-Hilaire in 1807, and originally assumed to be a sub-population, more recent genetic research has found that the individuals were, in fact, a separate species (Hekkala et al. 2009; Hekkala et al. 2011). The species, the Western African crocodile (*Crocodylus suchus*), could be the first in a series of crocodilian species split in response to genetic findings. Research into genetic variation of African dwarf crocodiles (genus *Osteolaemus*), for example, suggests that morphological variation displayed by geographically separated populations could, in fact, comprise three separate species (Eaton et al. 2009). The relatively localised speciation that appears to have occurred in Africa can, potentially, be attributed to the temporal persistence of the species.

Extant crocodilian species are divided into four different families; namely the Alligatoridae, Crocodylinae, Gavialidae and Tomistoma. Alligatoridae consist of two separate sub-families, the 'true alligators' (Alligatorinae) and the caiman (Caimaninae). The Crocodylinae family, or the 'true crocodiles', consists of 14 extant species, including the newly confirmed Western African crocodile, as well as the estuarine crocodile (*Crocodylus porosus*) (Brochu 2000; Hekkala et al. 2011). The Gavialidae family is represented by a single extant species of gharial (*Gavialis gangeticus*), a fish-eating crocodile with a slender snout and a bulbous growth around the nostrils (Lang et al. 2010). The Tomistoma family, often placed within the family Gavialidae, also contains a single fish-eating extant species (*Tomistoma schlegellii*) (Bezuijen et al. 1997).

The estuarine, or 'salt water', crocodile (*C. porosus*) is the largest extant reptile in the world and can reach a length in excess of 6 m in the wild (Britton et al. 2012). Its range stretches from Northern Australia, throughout Southeast Asia, and into Eastern India and Sri Lanka (Read et al. 2007; Campbell et al. 2010).

Estuarine crocodiles are semi-aquatic, although the majority of their time is spent in water (Campbell et al. 2010). They inhabit both fresh and salt water, as well as brackish estuaries (Lewis et al. 2013). Their euryhaline nature can be attributed to the possession of lingual salt glands, as well as possessing skin that is capable of withstanding a high osmotic gradient, providing a barrier when in highly saline conditions (Taplin & Grigg 1989; Kay 2004B). This adaptability has allowed them to hold both a large geographic range and persist throughout that range despite widespread hunting and persecution (Ross 1998). All crocodilians exhibit basking behaviour, the process of absorbing energy from direct sunlight, usually on banks, but occasionally on fallen trees and on logs (Grigg et al. 1998; Dinets et al. 2014). A crucial behavioural trait, basking ensures that individuals can hunt at night despite their ectothermic metabolism. Peak basking times are shortly before and after midday heat (Bourquin 2007).

Estuarine crocodiles reach sexual maturity at approximately 3.5 m (total length) for males and 2.5 m (total length) for females (Webb & Manolis 1989). Mating occurs in shallow water after an often elaborate courtship display (Garrick 1977). Males are highly territorial and will defend their territory aggressively against rival males (Lewis et al. 2013). Males display two separate mating tactics; 'fighting' (males who fight for a territory to win the right to mate) and 'sneaking' (individuals that do not hold strong territories but mate opportunistically) (Campbell et al. 2010). Dominant males will interrupt courtship between females and sub-dominant males (Lewis et al. 2013).

Crocodilians use a variety of vocalisations and signals to communicate with conspecifics (Campbell 1973). Beginning from inside the egg (see Section 2.3 below), vocalisations continue after hatching to indicate distress (Vergne et al. 2007; Vergne & Mathevon 2008), while adult signals range from territorial to courtship displays, with the establishment of territories preceding courtship rituals (Garrick 1977). Territorial disputes result in a wide array of passive and highly aggressive behaviours. Inflated posture is a common 'passive' behaviour and occurs when two males are establishing whether an encounter will end in

aggression; 'bellowing' (subsonic rumbling from the vocal cavity) is a commonly used behaviour to intimidate or taunt the opposing male (Garrick 1977; Senter 2008). Escalation of an encounter can lead to 'mock' biting of limbs and the tail, as well as 'head slaps' (a loud sound is generated when the head is slapped against the surface of the water) (Garrick 1977). Should escalating levels of aggression not prove decisive, physical aggression with an attempt to harm can be employed, this usually manifests as biting and can result in serious injury or death.

Estuarine crocodiles prey on a wide range of taxa, the identity of which shifts markedly as the individual matures (Magnusson et al. 1987). Crocodilians are ambush predators, generally waiting for their prey to come towards them (Campbell et al. 2010); they may, however, also scavenge a large proportion of their diet (Lindner 2004). For aquatic hunting, all crocodilians possess vibrational sense organs, known as integumentary sense organs (ISOs) (Jackson et al. 1996; Leitch & Catania 2012). *Crocodylus porosus*, along with all members of the *Crocodylus* genus, are covered with ISOs throughout their body enabling detection of prey located anywhere in the surrounding water (Jackson et al. 1996). The ISOs of members of the genus *Alligator*, on the other hand, are restricted to the cranium (Leitch & Catania 2012). The high number of ISOs found in *C. porosus* ensures that they are able to prey on a vast array of different species, including humans. While several species of crocodilian could, potentially, prey on humans, *C. porosus, C. niloticus* and *C. palustris* (mugger crocodile) are those most commonly responsible and responsible for over 90% of crocodile related human deaths worldwide (CrocBite 2014).

The legal protection of estuarine crocodiles throughout their range has resulted in an increase in mean size, as the majority of individuals hunted being large adults (Caldicott et al. 2005). This size increase, as well as an increase in overall numbers, has led to mounting number of attacks on humans. Often regarded as the most territorial and potentially most dangerous crocodilian, estuarine crocodiles inhabit mainly rural areas. This does somewhat limit the level of human-crocodile conflict (Caldicott et al. 2005). Nile crocodiles are, however,

the species with the highest human mortality rates, with 63% of attacks proving fatal (this is compared to the 50% mortality rate inflicted by Malaysia's estuarine crocodiles) (Sabah Wildlife Department 2010A). The vast majority of attacks occur during the wet season, with most attacks (81% in *C. porosus*) directed towards people either in or near the water (Kar & Bustard 1983). Attack data worldwide are underestimated as many attacks go unreported; this is a particular problem in developing nations and in areas where illegal immigration is high (Aust et al. 2009). Currently, the International Union for Conservation of Nature (IUCN) Crocodile Specialist Group keeps the most complete and up-to-date figures regarding human-crocodile conflicts, including the number of fatalities. Official figures within the state of Sabah note, within the period 2000-2011, 19 fatalities and 15 non-fatal attacks (Sabah Wildlife Department 2012).

The past 70 years have been uncertain ones for the estuarine crocodiles in Sabah, experiencing a large population crash during the mid- to late 20th Century (Whitaker 1984). Crocodiles became protected by law in Sabah in 1982, as well as being listed on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Sabah Wildlife Department 1997). This level of protection ensured that the unlawful killing of an individual would result in up to 5 years' imprisonment and a RM50,000 (~£10,000) financial fine. These efforts allowed a large scale rebound, with crocodile populations recovering to stable levels across the state (Sabah Wildlife Department 2010A). During the mid- 20th Century, the species was heavily exploited for its valuable skin deemed at the time to be the 'height of fashion' (Thorbjarnarson et al. 1992). The decline of the crocodile skin industry, coupled with the development of sustainable and responsible farming techniques, have allowed for a worldwide recovery of crocodilians over the past 30-40 years. This is due, in no small part, to the considerable efforts of the IUCN (International Union of Conservation of Nature) Crocodile Specialist Group, which has been managing populations worldwide since 1971.

2.2 Nesting ecology

Most extant reptiles are oviparous, reproducing by laying eggs, which develop independent of the female (Manson 2008; Tosun 2013). This reproductive strategy, widespread throughout the fossil record (almost all dinosaurs were oviparous (Farlow et al. 1995; Sander et al. 2011)) is retained not only by reptiles but also by all extant species of bird (Shine 2005). Reptiles lay a range of different clutch sizes, usually dependent not only on the size of the female, but also on the foraging behaviour of the species (Das 2010). All extant species of crocodilian, turtle, terrapin and tortoise are oviparous. In contrast to birds, oviparous reptiles ovulate and shell eggs concurrently in batches of four to six. This ensures that a clutch can be laid as a unit (Iverson & Ewert 1991). A wide range of clutch sizes are found even within taxonomic groups. For example, within the order Testudines (turtles), marine turtles lay in excess of 150 eggs compared to the spiny turtle (*Heosemys spinosa*), which lays only one or two (Herman 1993; Van Buskirk & Crowder 1994). Clutch size is often related to juvenile mortality, with species prone to high mortality rates compensating with large clutch sizes (Madsen & Shine 2006; Sander et al. 2011). Some reptiles choose to split their reproductive effort over several locations minimising the risk of complete loss by predation (Van Buskirk & Crowder 1994). Breeding frequency also varies between species and depends both on the age of sexual maturity, as well as juvenile survival (Shine 2005). The file snake (*Acrochordus arafurae*), for example, which takes four years to mature, then proceeds to produce a single clutch only once every four years (Madsen & Shine 2001).

For oviparous reptiles, one of the most important factors that influence hatchling success and survival is nest-site selection by adult females (Shine 2005). There are examples of optimal nest site selection within snakes (Brown & Shine 2006), turtles (Wood & Bjorndal 2000; Kolbe & Janzen 2002; Wilson 2005), lizards (Warner & Andrews 2002; Doody et al. 2006) and crocodilians (Somaweera & Shine 2012; Harvey & Hill 2003). Nesting of estuarine crocodiles usually occurs in marsh land, although this does vary depending on the available habitat in specific river systems (Webb et al. 1977). Nests generally consist of a central mound, usually constructed from mud or sand, with a

central excavation to hold the eggs (Webb & Cooper-Preston 1989). Egg-laying is completed within one hour and eggs are then subsequently covered by up to 30 cm of nesting material (Webb et al. 1977). Females are at their most aggressive during the nesting season, actively guarding the nests (Harvey & Hill 2003). Typically, nests are placed in remote locations, minimising the potential for human-crocodile conflict. Human activities such as egg collection, as well as female crocodiles being pressurised to nest closer to human-occupied areas, due to habitat loss and fragmentation, create increasing potential for conflict.

Meticulous nest-site selection is of particular importance in reptiles that exhibit temperature-dependent sex determination (TSD) (Janzen 1992; Roosenburg 1996; Shine 1999). By this process, gender ratios of a given clutch are determined by the internal nest temperature, both at the time of initial incubation and the temperature maintained throughout the incubation period (Valenzuela & Lance 2004). Found in a range of reptiles, including some lizards and turtles, TSD is uniformly present throughout all crocodilian species (Roosenburg 1996). There are three temperature-related gender patterns within TSD (Valenzuela & Lance 2004): 1) species exhibiting TSDII, include crocodilians, turtles and lizards, produce females at both high and low temperatures, and males at intermediate temperatures; 2) TSDIa individuals, such as marine turtles, produce males at low temperatures and females at higher temperatures, with a mid-range temperature producing a 50:50 sex ratio; and 3) TSDIb individuals which produce females at low temperatures and males at high temperatures, common to some lizards and crocodilians. Estuarine crocodiles exhibit TSDII (Webb et al. 1987; Lang & Andrews 1994; Valenzuela & Janzen 2001); low temperatures, between 28 °C and 30 °C, result in 100% female offspring, while temperatures of 31 °C, 32 °C and 33 °C produce 16%, 86% and 17%, respectively (Lang & Andrews 1994). Estuarine crocodiles differ from some other crocodilians, such as American alligators (*Alligator mississippiensis*), in that no single temperature can result in a sex ratio of 100% male (Webb & Cooper-Preston 1989; Lang & Andrews 1994).

While maternal protection of nests is relatively rare in reptiles, a number of crocodilian species do exhibit both nest defence and post-hatching maternal care (Harvey & Hill 2003; Vergne & Mathevon 2008). Evolution of parental care is likely to be linked to the need for female reptiles to assess and control the nest temperature, with increased presence leading to higher hatchling viability (Aubret et al. 2005). Some crocodilian species will stay with their hatchlings for a period spanning a number of months (Lewis et al. 2013). Predation is also a factor with both wild boar (*Sus barbatus*) and water monitor lizards (*Varanus salvator*) documented as estuarine crocodile nest predators (Cott 1971). The degree of maternal protection proffered to a nest depends on its location. Nests with a dense overhead cover, for example, lead to females guarding at close proximity while more open nests result in females guarding the nest from a distance.

Crocodile nests consist of a mound of mud and vegetation; depending on the species, these mounds average 1.5 - 2.8 m in width at the base, and are usually between 50 and 80 cm in height (Joanen 1964; Platt et al. 2006). Surrounding the nests will be a series of wallows which often form a water-filled moat around the nest (Webb et al. 1977). The egg cavity is usually located deep within the mound; the mean (±s.e) distance from the top of the nest to the egg cavity being 19.0 ± 0.6 cm (Webb et al. 1977). Egg shape and size vary between species of crocodilian but usually consist of hard-shelled ellipsoid shaped eggs approximately 8 -10 cm in length. Clutch sizes are species-dependent and vary between 30 and 70 eggs (Webb et al. 1977). Incubation times are often linked to environmental conditions, but typically require between 65 and 98 days from laying to hatching (Joanen 1964; Manson 2008; Hossain et al. 2012).

Hatching events are triggered by vocalisations of embryos; these calls stimulate a digging response in the female (Vergne et al. 2007; Vergne & Mathevon 2008). Pre-hatchlings are equipped with an egg tooth, located on the tip of the snout, to aid the cracking of both the inner-egg membrane and the hard outer-shell (Manson 2008). Hatchlings unable to break through the outer shell may be assisted by the female, a process where the un-hatched egg is taken in the jaws

of the female and gently cracked between the teeth (Pooley 1977). Once hatched, the majority of juveniles remain in the vicinity of the nest for an extended period, continuing to receive protection from the female. This phase of parental care can extend for a period of months (Lewis et al. 2013). Some hatchlings, however, disperse from the nest location soon after hatching, travelling distances of up to 1 km (Webb et al. 1977).

Post-hatching mortality is high (49%) although experimental data on wild individuals are limited (Brien et al. 2014). In areas where crocodilian populations are depleted, mortality rates are greatly reduced, with Webb et al. (1977) finding mortality rates of only 15.5% over the first two months following hatching. This is possibly due to reduced rates of probable infanticide by other adult individuals. Daily growth rates of newly hatched young (< 0.5 m length) vary between 0.01 and 0.08 cm per day, with rates increasing to 0.11 cm per day for individuals between 0.5 and 1 m (Anuar et al. 1996). In terms of weight, individuals less than 0.5 m in length gain 0.3-1.5 g per day, with those individuals between 0.5 and 1 m gaining 2.7-2.8 g per day (Magnusson & Taylor 1981; Webb et al. 1991). The diet of juvenile individuals is diverse and is dependent on local resource availability (Anuar et al. 1996); individuals less than 1 m in length generally feed on a combination of invertebrates (Crustacea, Hexapoda, Natantia, Reptantia) and small vertebrates (Anuar et al. 1996). Spatial ecology of nesting refers to the spatial organisation of nesting grounds, dealing specifically with random versus non-random distributions of nest sites, as well as, determining preferences for locations with specific environmental and habitat variables. Nesting behaviours throughout the Reptilia are highly diverse, and the spatial ecology of nest sites is as diverse as the class itself. Species range from being isolated, solitary nesters, exhibiting highly aggressive intra-species aggression, to females that aggregate at high densities and may even forgo foraging opportunities to achieve an ideal nest site (Graves & Duvall 1995; Lewis et al. 2013). Some species of reptilians build communal nesting systems; *V. panoptes*, for example, builds a series of interconnected burrows in which a number of females may lay eggs (Doody et al. 2014). This form of aggregate nesting behaviour appears to have ancient evolutionary links, with

strong evidence of some dinosaur species laying communally. There are two main hypotheses (Doody et al. 2009) as to why communal egg-laying behaviour evolves in a species. First, the "by-product hypothesis" argues that a lack of suitable nesting habitat can force conspecifics to nest in close proximity to one another. The second, the "sexual-selection hypothesis", implies that the locations of nests are a direct response to mating strategies. Especially common in highly polygamous species, communal egg-laying and nesting behaviour is believed to be a direct result of aggregations of females mating with a single or very few males. The spatially aggregated nature of females when conception takes place leads to the more "communal" appearance in nest spatial organisation; females tend to oviposit close to the site of conception. Communal nesting is found throughout the Crocodilia and has been identified in several species, including the American (*C. acutus*), the Australian freshwater (*C. johnstoni*) and the Nile (*C. niloticus*) crocodiles (Bourquin 2007; Kushlan & Mazzotti 1989; Tucker et al. 1998).

Solitary nesters exhibit strong nest site selection preferences and the fact that egg-laying is spatially isolated from other nests is both deliberate and considered (Escalona et al. 2009; Radder & Shine 2007). For species that are facultative, nesting both communally and solitarily, no differences have been identified in site habitat selection. This suggests that, for these species at least, habitat preference is not the driving force behind whether nests are positioned communally or not (Radder & Shine 2007). The benefits of communal-laying appear to be associated with embryo survival (Harris & Gill 1980), although survival odds do not seem to be related to predation rates. Somaweera et al. (2011), for example, suggested that there were equitable levels of dingo predation when communal versus solitary nesting tendencies were explored in the Australian freshwater crocodile (*C. johnstoni*). Solitary nesters may, in fact, be at an advantage when it comes to nest predation (Doody et al. 2009), as well as avoiding increased rates of disease transmission and competition for nest-building resources.

Crocodilia species, whilst all exhibiting a number of analogous nesting behaviour features, exhibit a wide range of behaviours in terms of nesting habitat and the type of nest constructed (see Table 2 in Brazaitis & Watanabe (2011) for differences between extant species). Despite differences in size, life history and physiological traits, all species appear to prefer nesting in freshwater environments, usually in wetlands or grasslands (Grigg & Kirshner 2015). Crocodilians, as a group, are fastidious in their nesting habits; desirable, and previously successful, nesting sites may be used annually (Platt & Thorbjarnarson 2010; Webb et al. 1983). Individuals, however, having begun nest construction, will often abandon the site mid-construction if one or more environmental variables do not meet the necessary 'standards'; these abandoned nesting attempts are referred to as 'test holes or mounds' (Brazaitis & Watanabe 2011; Grigg & Kirshner 2015; Somaweera & Shine 2012). This attention to detail suggests a definitive and considered approach to nesting; it is a cogitated approach to nesting common throughout the Reptilia.

The nesting of crocodilians is often remote; although, it can occasionally occur in close proximity to human settlement. This latter overlap increases the likelihood of conflict occurring between man and animal. Female crocodiles are generally thought to be far less aggressive towards humans than their male counterparts, however, during the incubation period, and shortly following the hatching of young, females can exhibit increased aggressive behaviour and have been responsible for numerous human injuries, and even fatalities (Brazaitis & Watanabe 2011; Caldicott et al. 2005). Female *C. porosus* generally guard nest sites (see Table 12.3 in Grigg & Kirshner (2015) for complete list of species exhibiting parental care); this can be at a distance, only returning to, and appearing at, the nest if a predator is detected (Webb et al. 1983). Males assist in the protection of nest sites in the spectacled caiman (*Caiman crocodilus*); in general, however, male assistance is generally rare amongst crocodilians (Grigg & Kirshner 2015). The level of protection or, in some cases, whether protective behaviour is displayed at all, varies between individuals (Grigg & Kirshner 2015).

Mound-nesting is one of two major nesting strategies employed by crocodilians; eight species opt for the somewhat rarer, and evolutionarily more primitive, 'hole-nesting' strategy (Greer 1970; Campbell 1972). Mound-nesters use a variety of nest substrates; *C. porosus* nests, for example, can be comprised of only sand, soil, leaf litter or grass, or a combination of these substrates, depending on local availability (Grigg & Kirshner 2015). As well as in substrate, crocodilian nests also vary in diameter. American alligator (*A. mississippiensis*) nests average 71.5 inches (181.6 cm) in diameter (Joanen 1964), those of the smooth-fronted caiman (*Paleosuchus trigonatus*) and the Siamese crocodile (*C. siamensis*), approximately 150 cm in diameter (Magnusson et al. 1985; Platt et al. 2006), and that of the estuarine crocodile (*C. porosus*), 175 cm in diameter (Webb et al. 1977). Species size does not appear to play a significant role in the size of the nest mound. The requirement of all species for ready access to permanent water sources (Harvey & Hill 2003; Somaweera & Shine 2012; Webb et al. 1983) limits nesting habitat. This necessity also plays a key role as one of the major causes of embryonic mortality, nest flooding (see below).

Two major factors are responsible for the majority of *in-utero* and neonatal fatalities of young crocodiles. Firstly, nest-based predators such as birds, lizards, snakes, rodents and insects, and even other crocodilians, have been reported to be nest- or crèche- (see Section 2.3) raiders (McNease & Joanen 1977; Somaweera et al. 2013). Secondly, as mentioned above, nest flooding is a major cause of juvenile mortality. Webb et al. (1977)'s study of nesting in the Northern Territory, for example, saw 17 of 45 identified nests flood, with all embryos inside them dying as a result of asphyxia caused by the reduced gaseous exchange across the shell surface (Grigg 1987). Females often return to nests and provided nest protection after flooding despite hatchling mortality (Webb et al. 1977). Local climate variables, such as precipitation and associated water flow, can, therefore, have a large bearing on nesting success and several crocodilian species (including *C. porosus*), in an attempt to mitigate such risks, construct floating rafts of vegetation. These, however, do also routinely flood (Campos 1993; Webb et al. 1983), but their close proximity to permanent water sources does allow the safe passage of the hatchlings, by female transport, to

communal crèche sites (Webb & Cooper-Preston 1989; Webb et al. 1977). The majority of hatchlings subsequently aggregate in such locations for a period of up to 2.5 months (Magnusson 1980A; Somaweera et al. 2013). Whilst nesting locations differ between species, *C. porosus* is widely reported to nest both in tidal swamplands and inland freshwater environments (Brazaitis & Watanabe 2011), these areas are generally flood-prone and flooding acts as one of the major factors in prenatal juvenile mortality.

Crocodylus porosus's nest consist of a mound of vegetation housing between 18-68 eggs (Grigg & Kirshner 2015; Hossain et al. 2012; Webb et al. 1977). These are incubated within the nest for 80-90 days (Brazaitis & Watanabe 2011). Seemingly obligative mound-nesters, *C. porosus* females do not appear to nest in any form of community structure, even when nesting habitat is scarce. Females will continue to exhibit aggression towards conspecifics throughout the incubation period (Lang 1987). Nests are usually constructed within 20 m of permanent water (Webb et al. 1983), and are most often found in areas of swampland and riverine habitats (Magnusson et al. 1978). Mangrove and sedge plains are considered the least suitable habitat for *C. porosus* nesting (Harvey & Hill 2003; Magnusson et al. 1978). Mangrove habitat lacks sufficient open areas and necessary nest building vegetation, whereas sedge plains are often located too far from permanent water. Swamplands appear to be the most universally utilised areas and are the prevalent habitats within much of the estuarine crocodile's range.

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2.3 Habitat Fragmentation

An accurate estimation of the number of species living on Earth remains unknown; however, there is a general consensus that there are a total of 1×10^7 – 1×10^8 separate extant species (Pimm et al. 1995). These species are not uniformly distributed across the globe (Brooks et al. 2002; Myers et al. 2000); areas identified as exhibiting higher than average species densities having

obvious conservation importance. Globally, in 2000, 25 areas were designated biodiversity 'hotspot' regions. These cover a range of environments and habitats and were defined as containing at least 0.5% of the world's plant species; their designation attempted to ensure effective partitioning of conservation funding and other resources (Myers et al. 2000). Since their designation additional hotspots have been identified, with the most recent addition of the forests of eastern Australia, the 35th hotspot, being proposed by Williams et al. (2011).

Southeast Asia is home to four separate hotspots and is a 'true reservoir' of biodiversity. It is, however, also an area whose forests are undergoing more rapid conversion than any other part of the planet (Sodhi et al. 2004); the region is currently losing its forest cover at twice the rate of Africa and almost thrice that of Latin America (Achard et al. 2002). Estimates by Sodhi et al. (2004) suggest that 75% of forests within Southeast Asia could be lost by the year 2100, with a 40% reduction in biodiversity (revised to be between 13% and 85% by Sodhi et al. (2009)). Sundaland is one of Southeast Asia's four biodiversity hotspots (Myers et al. 2000; Sodhi et al. 2004). It is currently experiencing heavy conversion with an average rate of 1.75% decrease in forest cover per annum across the region (Brooks et al. 2002). Rich in endemic flora and fauna, the region is particularly rich in herpetofauna, with an estimated 60% of reptiles and 80% of amphibians being endemic to the region (Sodhi et al. 2004). Sundaland has the second highest percentage of endemism of any of the global hotspots, with 5% of global endemic plants (Myers et al. 2000). This combination of rapid deforestation, land conversion and high endemism has the potential to lead to rapid extinction rates. Currently, 43% of endemic mammals found throughout Sundaland are classified as endangered (Brooks et al. 2002).

During the 20th Century, the large-scale conversion of forest into agricultural use has been widespread; it is Southeast Asia that has experienced the fastest conversion rates (Sodhi et al. 2004). This conversion has continued into the 21st Century and in the period 2000 – 2012, Malaysia, along with Cambodia, Côte d'Ivoire, Tanzania, Argentina and Paraguay, has experienced one of the highest

reductions of forest cover (Hansen et al. 2013). The main driver of this conversion over this period has been oil palm (*Elaeis guineensis*) the world's most rapidly expanding crop (Fitzherbert et al. 2008). Originating from western Africa, oil palm is now grown commercially in at least 16 countries (Wahid et al. 2005) and, as of 2012, over 17 million ha of oil palm are being cultivated worldwide, an increase of 7.13 million ha (41.7%) since 2000 (Abram et al. 2014).

Sabah has experienced widespread land conversion over the past 50 years, fuelled by both the timber trade and the expanding palm oil industry (Abram et al. 2014; Reynolds et al. 2011). Timber was seen as a quick pay-off from the land but, with no repeat pay-off due to period needed for regrowth of high value trees, along with limited available land, this type of land conversion was only tenable in the short- to medium-term. In 1979, Sabah's income from the timber trade was estimated at US$1.1 billion (~£725 million) (Toh & Grace 2007). In reality, these revenues translated into a reduction in the overall cover of primary forest of Class II (commercial forest reserves) production from 98% to 15% between 1970 and 1996 (Toh & Grace 2007). This land conversion has resulted in large-scale habitat fragmentation, including the limiting of the movement and dispersal of many species, as well as increasing the potential for human habitation of areas that were historically remote. This, in turn, increases the potential for human-wildlife interactions. As of 2011, Sabah was permanently cultivating 1.43 million ha (19.3% of the total land area of the state) of oil palm. Expected to rise to 2.1 million ha by 2025, the majority of conversion is most likely to happen in the eastern lowland floodplains, including that of the Kinabatangan (Abram et al. 2014).

Malaysia and Indonesia are the world's largest producers of oil palm; Malaysia has already devoted over 6 million ha of land to its production (Koh & Wilcove 2007). This is projected to reach 15 million ha by 2020 (Hai et al. 2001). By 2003, oil palm plantations accounted for 86% of all cultivated land in Sabah (Toh & Grace 2005). Peninsula Malaysia was the first to experience large-scale palm oil exploitation, with Sabah experiencing rapid land conversion during the

late 1990s (Hai et al. 2001). The incentive for this rapid expansion was the increase in profitability stemming from more efficient growth of oil palm cultivars producing increased yields. In comparison with other highly profitable "cash crops" such as soya bean, oil palm is far superior in terms of yield per hectare (Wahid et al. 2005). Oil palm produces 3.30 tonnes ha^{-1} $year^{-1}$ of oil, compared to soya bean, which produces 0.46 tonnes ha^{-1} $year^{-1}$ (Wahid et al. 2005). A major drawback with oil palm production, however, is that the early years after planting generally produce negligible or no yield (Wahid et al. 2005). With selective breeding and good plantation, trees can begin to produce large yields in as little as 2.5 years (Wahid et al. 2005). Worldwide oil palm production is rising by 6.6 - 9% per annum, making it the largest growing agricultural sector (Fitzherbert et al. 2008; Wahid et al. 2005).

The agricultural success story of oil palm has been intrinsically linked to the vanishing habitat of much of Southeast Asia's endemic wildlife. Koh and Wilcove (2007) cited oil palm as the premier threat to biodiversity across the region and this is testament to the scale of the challenge that can occur when highly profitable business comes into conflict with the natural world (Fitzherbert et al. 2008). To date, most agricultural conversion in Southeast Asia has occurred in optimal-yielding areas but future expansion will, and has already begun to, explore sub-optimal land in terms of oil palm yield; this will increase pressure on areas of agriculturally-ideal lowland forest dedicated as wildlife sanctuaries (Abram et al., 2014). Unfortunately, these agriculturally valuable areas are also the most important areas for biodiversity (Fitzherbert et al. 2008).

The effects of habitat fragmentation on crocodilians are, at a glance, less dramatic than other, terrestrial, predators. Impacts on prey abundance, as well as the secondary effects of anthropogenic expansion, for example, over-fishing, have resulted in declining populations of many higher order predators (Hinlo et al. 2014; Mauger et al. 2012). The urbanisation of remote areas has resulted in far more ready access to exploitable resources, resulting in the harvesting of millions of crocodilians during the 20th Century (de Thoisy et al. 2006).

Persecution of large carnivores in the 19th and 20th Centuries coincided with rapid rates of anthro-expansion worldwide (Woodroffe 2000). A classic example of an apex predator extirpated during that period is the thylacine (*Thylacinus cynophalus*) (Paddle 2002). This species became extinct as a result of persecution relating to attacks on livestock, with a bounty scheme offering rewards for dead thylacine (Paddle 2002). Many large predators, with relatively isolated populations, did, however survive the 20th Century (Grayson 2001). Increasing habitat fragmentation brought these predators into more frequent contact with humans, creating conflict on both sides (Distefano 2008). The Sumatran tiger (*Panthera tigris sumatrae*) is one such predator, responsible for 146 deaths in Sumatra during the period 1978 - 1997 (Nyhus & Tilson 2004). Human fatalities represents only a fraction of conflict events occurring worldwide, for example, in Alberta, Canada during 1982 – 1996, grey wolves (*Canis lupus*) were responsible for a reported 2,086 fatalities among livestock (Distefano 2008). These 'human interest' costs represent a huge barrier to conservation of predators. The protection and management of apex predators such as crocodilians requires the support of local people. To achieve this, people must understand more about the species. Understanding more about crocodilian behaviour and ecology can help educate people about their life history, as well as to identify areas and times which are more likely to result in conflict.

2.4 Remote Tracking

Remote tracking of animals has revolutionised the way in which much of behavioural, landscape and conservation biology is conducted in the field. The ability to know the exact location of an animal without directly observing the individual allows for minimised bias and the preservation of 'natural behaviour' (Rodgers 2001). This 'observer-removed' monitoring style allows for larger scale, longer-lasting studies (Seegar et al. 1996). As, however, with most new technology, inception costs are high; equipment units are usually expensive and may be a limiting factor in determining sample sizes (Franklin et al. 2009). In general, however, as the technology develops and becomes more frequently used, costs tend to become lower and the technology can become financially

viable for a wider range of users. The quantity and quality of data that can be remotely collected enable a greater understanding of home ranging, especially for animals with larger ranges. Furthermore, a detailed, fine-scale understanding of habitat utilisation can result in more effective management strategies (Hulbert & French 2001; Franklin et al. 2009).

Remote monitoring of animals began in the late 1960s with Very High Frequency (VHF) tracking. Many early studies were focussed on American alligators (*A. mississippiensis*). Early tracking units were bulky and heavy, and *A. mississippiensis*, with its large size and weight, was an ideal study species. Joanen and McNease (1970) VHF-tagged five female alligators and were able to determine both home range and nesting habits during tagging periods ranging between 15 and 115 days. Two years later, this study was extended to look at movements of adult males; units were attached on a collar consisting of rubberised fabric (Joanen & McNease 1972). Another early study focussed on VHF-tracked adult American alligators, assessing seasonal movements between 1976 - 1977 (Goodwin & Marion 1979). Despite high levels of suitability for tag placement, the first satellite telemetry on crocodilians was still another 25 years away.

VHF-tracking requires constant monitoring of the animal, albeit at a distance, with triangulation, the process of identifying the location of a signal from three or more surrounding points, necessary to determine an individual's location reliably (Tomkiewicz et al. 2010). Whilst revolutionary in terms of home range estimations, it is a labour-intensive method of assessing home range. Animals can be 'lost' after collaring due to misjudgements of home range size or collar failure (Rodgers 2001; Sandgren 2012). The technology does, however, allow for more consistent direct behavioural observations (Baird et al. 2002; Chilvers et al. 2005) and allows for the animal to be tracked irrespective of time. Having established itself globally as the main method of individual monitoring for more than 20 years, VHF-tracking remains in use in many studies, often in conjunction with satellite technologies (Read et al. 2007; Alfred et al. 2012).

Although VHF-technology revolutionised the field at the time of introduction, the inherent limitations of the methodology catalysed the development of the next generation of tracking devices. This manifested itself as satellite tracking. Early satellite tracking devices were large, bulky, units that worked using the Advanced Research and Global Observation Satellite (ARGOS) system of satellites (Seegar et al. 1996). This system, still in operation, allows for geographical fixes to be established with an accuracy in the region of ± 1 km (Campbell et al. 2010). In addition, bulky electronics and inefficient batteries meant that smaller animals were not suitable candidates for satellite tracking. The system requires the user to purchase certain time frames during which satellite points are to be collected. One early study examined the ranging behaviour of the wandering albatross (*Diomedia exulans*). While the bird's large size and large home range were compatible with using the ARGOS system (Walker et al. 1995), units had to be retrieved in order to access the stored data. Heavily utilised in the tracking of Australian crocodilians during the past decade, the ARGOS system has been chosen for its reliability and coverage. Most of these studies have, however, focussed on large-scale ranging of individuals and, in particular, their association with the oceans of Northern Australia (Read et al. 2007; Campbell et al. 2010; 2013).

During the 1990s and 2000s, the USA-owned, Global Positioning System (GPS) group of satellites became increasingly more heavily used for satellite tracking (McCallum 2000; Augustine et al. 2011). The expensive nature of ARGOS systems, paired with the relatively high inaccuracy of the data accrued, led to this expansion (Campbell et al. 2010). GPS-satellite tags allow for inaccuracies measured in meters rather than kilometres (McCallum 2000), making them considerably more applicable for studying animals with small home ranges, as well as examining aspects such as habitat use and reactions to both landscape and anthropogenic influences. The Iridium Satellite Constellation, a group of 66 active communication satellites, allow for two-way transfer of data, and the low orbiting satellites provide a high level of coverage with very high accuracy. Iridium's satellites perform mostly Northern Polar orbits making them most effective in the Northern Hemisphere (Tomkiewicz et al. 2010).

As remote tracking technology has advanced, tracking units have became both lighter and easier to fit, with many different attachment mechanisms devised for working with a wide range of organisms (Rodgers 2001). Franklin et al. (2009) examined the efficiency of these different attachment techniques within crocodilians. The inherent problem with generating a satellite-validated location 'fix' is the large draw on battery voltage; transmission of this 'fix' to a base station or monitoring network compounds the draw on the energy source further. This challenge has catalysed a range of different solutions. Most currently-used satellite devices can be split into being dependent on one of four main data transmission methods (Rodgers 2001): in the first, data are transmitted back to the satellite using two-way data transfers. These data can then be downloaded to a central server and uploaded onto the Internet to be globally accessible (Rodgers 2001). The second method uses a locally-sourced cellular phone Subscriber Identity Module (SIM) card and transmits the data as a Short Message Service (SMS) message (Tomkiewicz et al. 2010). A base station picks up data in the same way that cellular phones pick up text messages. The drawback for this method is that the animal being observed must inhabit an area with widely available cellular service. Currently being trialled, the third approach is exploring a technique of satellite tags suitable for smaller animals (Tomkiewicz et al. 2010). This method requires the download of logged data using a hand-held base station directly from the tag. The researcher must reach a predefined proximity from the animal, at which time the base station will download the points from the collar. This process requires significantly less (compared to the first method) battery power for transmission, allowing units to be much smaller in size whilst still providing good tag longevity. The final method is referred to as a data logger which stores all satellite locations and the data can only be retrieved once the animal and tag is recaptured (Rodgers 2001; Tomkiewicz et al. 2010). Each of these systems has advantages and disadvantages associated with its use; the researcher has to determine which method proves most suitable for their individual research questions and needs. This diversity of available options does, however, allow for an ever-increasing wide range of organisms to be classed as 'suitable for satellite tagging'.

Various additional methods have been formulated to deal with the challenge of battery drainage when tracking animals; the most ingenious is probably the inclusion of solar-powered cells, casing the upper side of the tag (Monsarrat et al. 2013; Higuchi & Pierre 2005). This technique is reserved for animals that have access to direct sunlight for extended periods, for example, sea birds, travelling long distances across open water (Higuchi & Pierre 2005). The introduction of solar-powered satellite tags has enabled the mapping of these migration routes; the addition of the solar component means that a much lighter and smaller unit can be produced. Another benefit of solar panels is to reduce the overall weight of the tag; a matter of paramount importance when considering tagging methods on species that conduct long flights. This technology also has potential applications in marine environments, where long-term movements require a tag that is capable of recharging whilst animals surface (Rodgers 2001).

Satellite technology was not reported to have been utilised on crocodilians until 2007 (Read et al. 2007), with VHF-technology still being used in crocodilian research as recently as 2004. Kay (2004B) used VHF technology to track the movements of 16 crocodile individuals in the Northern Territory, Australia. Until then, most home range estimations in Australia had been approximated using mark-recapture techniques. Whilst successful, Kay's study required considerable tracking energy expenditure on the part of the researchers; it also documented the now widely used nuchal plate attachment technique. By attaching the unit to the nuchal plate (situated at the rear of the skull), the tracking units are above the waterline for the maximum amount of time, enabling the greatest number of data fixes and transmission. Other attachment methods have also been explored such as to the tail scute (Strauss & Botha 2008).

The need to develop satellite technology in crocodilian tracking has been argued to be essential for crocodilian management and conservation (Kay 2004A). Read et al. (2007) reported the first successful satellite tagging of

crocodilians, with the results showing the ability of these animals to travel large distances through open water. Shortly after this study, other techniques were explored including the attachment of time-triggered release of data loggers to animals. In this novel technique, on being jettisoned the unit floats using an attached balloon inflated by a small gas canister (Franklin et al. 2009). The monitoring time for any given tag using this technique is, however, relatively low. One technique that has not yet been explored in the satellite tagging of crocodilians is the use of remote download tags. This technology requires being able to locate the individuals at least once every few weeks to download data; not always feasible for long-ranging and sometimes unpredictable individuals, displaying erratic movement patterns, such as in Northern Australia. Past studies in the home range analysis of crocodilians have been metaphorically one-dimensional, mainly because of limitations in ARGOS technology. The inability to resolve the location of a fix to less than ± 1 km has meant that measurement of fine scale habitat utilisation has hitherto not been feasible. By using the Iridium satellite system, data collected allow for more precise movement analysis, as well as the introduction of more localised activity patterns and time-budgets (Tomkiewicz et al. 2010). Given that Iridium has the capacity for multidirectional data transfer it has been heavily used for mobile communications and is the main satellite system used by Motorola™ (Maine & Devieux 1999). This increased level of accuracy paves the way for more in-depth home range analyses, including analysis of habitat use within home ranges.

Previously, satellite-tracking technology has been effectively used to establish baseline crocodilian movements and to understand certain behavioural traits, such as homing in translocated crocodiles (Read et al. 2007). This current study seeks to explore more closely, site-use and activity patterns, both over the short- and long-term, in Bornean estuarine crocodiles. In addition, the study also explores the movements of crocodiles in a very different habitat compared to where previous studies have examined. The need for research in this region is considerable with many reported, and probably many more unreported, attacks occurring in Sabah alone. In addition, behavioural differences between

equatorial, rainforest dwelling crocodiles, and those of Northern Australia, are unknown. The study seeks to understand movements of adult crocodilians within a fragmented ecosystem.

2.5 Genetics

Crocodilians are an ancient order with little in the way of morphological change having occurred since their appearance some 140 m.y.a. (Fig. 2.1) (Janke et al. 2005). There are relatively few distinctions to be made, at least morphologically, between the Crocodilia and the Pseudosuchia, which appeared some 235 m.y.a. (Janke & Arnason 1997; Nesbitt 2003). The genetic reasoning behind this perceived lack of evolutionary development suggests that within crocodilian genomes, mutation rates are only approximately 25% those of birds and far slower than the majority of vertebrates (Green et al. 2014). This slow mutation rate could be responsible for the low number of differentiated species despite crocodilians' global distribution.

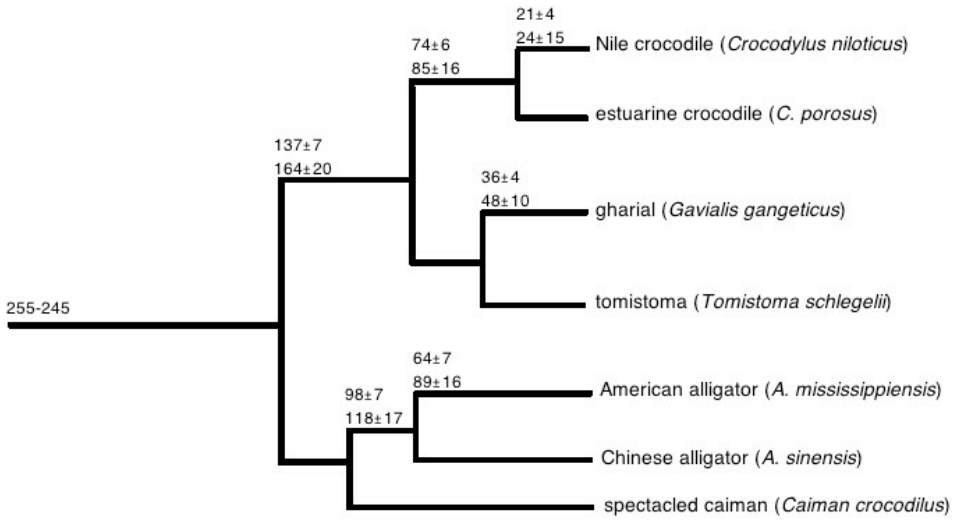


Figure 2.1. Phylogenetic tree displaying upper and lower boundary divergence (years ±s.e) times between species. Adapted from Janke et al. (2005).

Currently, there are 24 recognised extant crocodilian species worldwide (see Section 2.1) but as mentioned, genetic analysis of crocodilian populations has the potential to split existing species further. Evidence from Eaton et al. (2009), following the identification of species level divergence, suggests the presence of

a Congo basin dwarf crocodile (*Osteolaemus* sp.) and a separate western African species. The impact of genetics on crocodilian taxonomy, whilst important, represents only a tiny fraction of the potential of genetic research into crocodilians.

As well as being of direct biological and conservation relevance, crocodilian genetics are of serious commercial value in relation to the scale of global crocodile farming. Crocodilian farming is a developing industry, with an Australian value of AUD$8.8 million (~£4 million) in 2004 (RIRDC 2005), rising to AUD$15 million (~£7 million) by 2014 (RIRDC 2014), with 2011 worldwide trade estimated to be in the order of 1.36 million skins (Caldwell, 2013). Given the obvious financial incentives, work on the 'genetic improvement' of crocodiles has been relatively prolific, with phenotypically-superior crocodile individuals being selected as preferential breeders (Jaratlerdsiri et al. 2012). Phenotypes that are more disease resistant, together with other phenotypic advantages such as increased growth rates, are estimated to be worth AUD$324 (~£154) per pair per annum (RIRDC 2005). Given the obvious financial benefits, a focus on the full genome sequencing of a large range of crocodilians has become a priority amongst crocodilian geneticists and has led to the formation of the International Crocodilian Genomes Working Group (ICGWG). The ICGWG has focussed on the genome sequencing of three species in three families, namely the American alligator (*A. mississippiensis*), the estuarine crocodile (*C. porosus*) and the gharial (*Gavialis gangeticus*) (crocgenomes.org). Additionally, crocodilians have also become the focus of research into new, innovative antibiotic drugs. For example, the American alligator (*A. mississippiensis*) has been shown to harbour a range of broad-spectrum antibiotic peptides, largely isolated from the serum (St John et al. 2012). This compounds their commercial value. Despite the importance of immunogenetics, understanding genetics of wild populations is of equal importance, potentially allowing for better management strategies.

Landscape genetics is a relatively new concept, and aims to collate genetic, spatial and statistical methodologies to understand more fully how the ecology

of a particular species can influence the species's population genetics (Storfer et al. 2007). A number of crocodilian species, including *C. porosus* and *C. mindorensis*, are able to live sympatrically, albeit occupying similar niches (Platt et al. 2009; Pomares et al. 2008; Villamarin et al. 2011). This sympatric existence can potentially lead to levels of hybridisation between distinct species, such as has happened with *C. moreletii* and *C. acutus* in Central America (Ray et al. 2004; Rodriguez et al. 2008). Inter-species hybridisations are most prevalent in the Americas, where there exists the greatest number of sympatric species. Repeated introgressions with the sympatric *C. moreletii* have resulted in very high levels of hybridisation with the already endangered *C. acutus*; the result being in the existence of very few populations of genetically pure individuals of *C. acutus* (Machkour-M'Rabet et al. 2009). There is however, also evidence of hybridisation between genetically distinct populations of the same species (González-Trujillo et al. 2012).

As mentioned in Chapter 1, the vast majority of crocodilian populations experienced a severe population bottleneck during the mid-late 20th Century (Thorbjarnarson 1998). To date, examination of *C. porosus* genetics following this perturbation has presented little or no evidence of an associated genetic bottleneck (Gratten 2004; Russello et al. 2007). This also appears to have been the case for a range of other crocodilians across the globe; a lack of a recent bottleneck has also been demonstrated in *C. niloticus* (Flint et al. 2006) and *C. acutus* (Rodriguez et al. 2008). Glenn et al. (2002) did, however, find evidence of a more ancient bottleneck occurring in *A. mississippiensis* at around 21,000 years before present.

One of the largest genetic studies on *C. porosus* wild populations focussed on a small isolated population of individuals on the island nation of Palau (Russello et al. 2007). A single haplotype from 39 individuals was matched in samples of individuals in Western Kalimantan, Southern Borneo (Russello et al. 2007). Along with various studies on captive populations (Gratten 2004; Luck et al. 2012), Russello et al.'s study suggests that the entirety of the sampled demographic of *C. porosus* belong to a single Evolutionary Significant Unit (ESU

- Gratten 2004). This infers that the conservation of the species should be focussed at a species level rather than widespread efforts to preserve small remnant populations. However, these findings should be tempered with caution as only a small subset of regions harbouring surviving populations have been sampled genetically. This leaves the possibility that other regions may contain individuals belonging to a separate ESU.

In zoological terms, kinship refers to a direct blood relationship that can be the result of either sexual or asexual reproduction. The identification of relatedness or parentage between individuals has many applications across a range of species and is of particular interest to the crocodile farming industry (Isberg et al. 2004). Parental relations in crocodilians are complicated by the fact that many clutches of eggs consist of the progeny of multiple fathers. Multiple paternity has been documented in a number of species including *C. porosus* (Lewis et al. 2013), *A. mississippiensis* (River et al. 2001) and *Caiman latirostris* (Amavet et al. 2008). The presence of what are often referred to as 'sneaky males', those usually sub-dominant males that mate with females surreptitiously (Lewis et al. 2013). This shows that differing behavioural strategies can have an overall impact on the population genetics of a population, with the inclusion of often sub-dominant males whose genetic material might otherwise not be present in the gene pool.

2.6 Conclusions

Crocodilians are evolutionarily ancient apex predators, with serious human-conflict implications in an ever-changing world. Large-scale habitat conversion and anthropogenic expansion have brought people into closer proximity with these predators. Their commercial importance, coupled with their impressive body forms, does, however, mean that they are also subjects of concern in a conservation context. Improving our ability to protect these populations demands a better understanding of how they are utilise the landscape, both in terms of aquatic and terrestrial resources. Technology has a role to play in unearthing behaviours of this otherwise cryptic guild. A combination of high-resolution data, together with increased knowledge of the species demographic

history, is essential in initiating and developing an effective and achievable management plan.

Chapter 3 – Home Ranging and Anthropogenic Influences of the Estuarine Crocodile (*Crocodylus porous*) in Sabah, Malaysia.

3.1 Introduction

Crocodilians represent a family of aquatic predators unrivalled in their evolutionary longevity (see Section 2.2), as well as their adaptability to changing environmental conditions. Crocodilians have, by-and-large, persisted; there has only been one known species extinction, that of *Voay robustus*, directly linked to anthropogenic intervention (Bickelmann & Klein 2009). That the remainder of extant species have survived to the present day is testament to crocodile adaptability to rapidly changing environmental circumstances, both in terms of climate as well as anthropogenic alteration. Despite this versatility, the rate of habitat alteration, coupled with hunting pressures throughout the 20th Century, has brought many crocodilian species to the brink of extinction (Thorbjarnarson et al. 1998). Careful management has ensured the recovery of a number of these species, however, this recovery presents a new range of population management challenges in a world where anthropogenic expansion is redefining the word "remote".

Human-wildlife conflict is both a serious and an escalating issue (Distefano 2008). In its most fundamental sense, human-wildlife conflict can be described as an action of an animal that harms the general interests of humans. Madden (2004), however, recognised that the problem is far more complex than the issue of attacks on people and livestock. Human-wildlife conflict affects a wide variety of people from differing geographic and socio-economic situations; it is this fact that makes the issue a truly global one. Globally, six species of crocodilians are deemed to pose a direct threat to human life; the estuarine crocodile (*C. porosus*), the Nile crocodile (*C. niloticus*), the mugger crocodile (*C. palustris*), the American crocodile (*C. acutus*), the black caiman (*Melanosuchus niger*) and the American alligator (*Alligator mississippiensis*). Other crocodilian species are also credited with attacks, but generally, within these species, conflict with humans is extremely rare (Sideleau & Britton 2012). The reality of

conflict with species that have the potential to cause harm, both to people and their livestock, creates complex management practice issues; these are further complicated when the species in question is threatened with extinction (Woodroffe et al. 2005). Crocodilians pose a credible threat to human life and, with the species listed above credited with multiple human attacks. The fact that, as of 2014, seven crocodilian species are considered critically endangered on the International Union for Conservation of Nature (IUCN) Red List, and a further four vulnerable, means that management practice is rarely simple and must take a range of factors into account (IUCN Red List 2015). Conservation practice is further complicated by the fact that many species are considered commercially viable and provide income through both skin and, to a lesser extent, meat industries. While commercial crocodile farms harbour many critically endangered species, in multiple cases these genetic refugia have been compromised through both intentional and non-intentional hybridisation (Fitzsimmons et al. 2002). Commercial exploitation throughout the 20th Century was a leading cause of population decline in many crocodilian species (Fukuda et al. 2011; Hinlo et al. 2014; Stuebing et al. 2006; Russello et al. 2007). This commercially-mediated decline was particularly stark within estuarine crocodiles whose previously populous wide distribution was decimated resulting in a 'endangered' IUCN listing in 1982 (Fukuda et al. 2013). Rapid population declines have the potential to cause long-term deleterious effects, including the well-established genetic implications of inbreeding (Kay 2004B; Keller & Waller 2002). Impacts on the ecology and behaviour both during and after population recovery are, however, ill-understood.

The estuarine crocodile is the largest of the extant crocodilians, reaching in excess of 6 m in the wild (Britton et al. 2012). Its secretive nature means that despite an overlap in habitat use, the majority of large individuals seem to avoid conflict with humans (Kar & Bustard 1983). It is, however, still responsible, annually, for multiple deaths throughout its range and is thought to have been responsible for 461 recorded deaths worldwide since 2000 (CrocBite 2014). Understanding the relationship between what is, undoubtedly, a dangerous predator and human beings is of increased importance following expanding

crocodile numbers and human populations throughout the estuarine crocodile's range (Fukuda et al. 2011) (see Chapter 2). Mitigation of attack risk requires not only education and vigilance on the part of the local people, but also an understanding of how large, potentially dangerous, individuals utilise their habitats.

Territorial behaviour is often cited as a cause of human-crocodile conflict, as attacks with no, or only partial, consumption of victims are commonplace (Caldicott et al. 2005; Sideleau & Britton 2012). Territoriality is a dominance behaviour designed to acquire and/or maintain an evolutionarily beneficial spatial location. It remains unclear as to whether territorial aggression is primarily responsible for human attacks; Caldicott et al. (2005) suggested 89% of attacks have been reported as being perpetrated for nutritional gain although territoriality could also have played a role in some of them. Although male crocodilians are, in general, considered to be highly territorial, with species-specific degrees of individual territoriality suggested for different species (Lang 1987), very limited research exists on this aspect of crocodile behaviour. Given the lack of congruency within the literature, understanding if and how 'active' territoriality is occurring within a given habitat has the potential to increase understanding of habitat utilisation, predictions on prey availability, access to females, as well as enable development of management practice with a particular focus on anthropogenic attack avoidance.

Satellite-informed data analysis has the potential to provide unparalleled clarity regarding home ranging as well as increased understanding of important behavioural traits, such as the role of territoriality and how dominance hierarchies can influence crocodiles' aggression levels. Essential resources required for individual crocodilians vary between species but are diverse depending on habitat and individual requirements; resources are often associated with nesting and reproductive requirements. *Crocodylus porosus* is cited as being among the least tolerant towards rivals, defending territories perennially (Lang 1987). However, in Australia, male estuarine crocodiles have not appeared to exhibit specific spatial exclusion indicative of territoriality

(Brien et al. 2008; Kay 2004B), with males moving large distances to enhance female encounter rates (Kay 2004B). More recent findings, also in Australia, suggest that acoustically tagged individuals were, by and large, restricted to distinct stretches of river (Campbell et al. 2010).

There is a paucity of studies examining the fine scale movements and territoriality of crocodilians; only by increasing our knowledge, and understanding of movement patterns and home range utilisation can the complex issues surrounding population control and trophic ecosystem stability be addressed. Managing populations effectively to ensure not only the safety of local people but also avoiding large scale population instability is of paramount importance, not only within crocodilians but when dealing with potential human predators worldwide (Woodroffe et al. 2005).

The use of satellite monitoring for crocodilians is relatively novel, with the first recorded tracking taking place in 2003, displaying homing behaviour and oceanic travel (Read et al. 2007). Further analysis of these data suggested the utilisation of favourable ocean currents to expedite long range travel (Campbell et al. 2010A). Examination of home range strategy became possible with the new levels of accuracy available from satellite units. Campbell et al. (2013) reported a mean (±s.e) accuracy of 12.1 ± 1.1 m in location points used for analysis, allowing a level of fine scale analysis previous units were unable to provide. While analysis of home range utilisation has previously entailed the use of minimum convex polygons (MCPs) and kernel utilisation densities (KUDs) (Campbell et al. 2013; Kay 2004B; Tucker et al. 1997), to understand the inter- and intra-territory mechanics of adult crocodilians, more comprehensive analyses on a finer spatial scale are still required.

Home range analysis has found that male *C. porosus* exhibit one of two behavioural strategies: site-fidelic (those individuals confined to a small discrete stretch of river) or nomadic (further ranging with a lack of apparent territory). The presence of these two strategies appears to be linked to individual size, with site-fidelic and nomadic males averaging 4.1 m and 3.6 m

in length, respectively (Campbell et al. 2013). Home range sizes of these individuals were measured using KUDs and found to range from 7.1 to 72.5 km^2, with nomadic males straying much further from their core range than would be expected for smaller-sized individuals. Females, often caught less frequently in trapping studies, exhibited similar variation, but on a smaller scale than males. These observations provided base-line data for *C. porosus* home ranges and, for the first time, examined intra-sexual variation in behavioural traits, and fine-scale home range utilisation.

Sabah, the eastern-most state of Malaysia, located in the North East of the island of Borneo, provides excellent habitat for estuarine crocodiles, with large rivers, an equatorial climate, and a diverse prey base (Turner & Foster 2008). Crocodiles in Sabah have endured a tumultuous half-century. Following their listing, in 1982, as a Schedule I species (killing of crocodile punishable by fine and up to five years imprisonment, unless in self-defence or for licensed scientific research), initial surveys were carried out in association with the World Wildlife Fund (WWF) (Whitaker 1984). These surveys yielded very low crocodilian densities of just 0.21 km^{-1}. In the intervening 30 years, there has been an undoubted recovery although with the most recent survey carried out some 14 years ago survey data require updating (Sabah Wildlife Department 2010A). Carried out across seven of Sabah's major rivers, the 2002 survey yielded a density of 2.27 km^{-2}, a more than ten-fold increase over a 20-year period (Sabah Wildlife Department 2002). A management plan was designed for the Sabah crocodilian population in 2010. At this time, it was estimated that, throughout the state, some 13,000-15,000 individuals were living, including a viable percentage of breeding adults, indicating the potential for further population expansion. Furthermore, the prospect of the ever-expanding eco-tourism sector in Sabah places enhanced value of wild individuals. The 2010 Management Plan cited the state's potential annual revenue from crocodile-based tourism streams as RM (Ringgit Malaysia) 27 million per annum (~£4 million).

Whilst there is undoubtedly under-reporting of both fatal and non-fatal attacks in Sabah owing to delicate socio-political factors involving immigration, there have still been a total of 25 attacks reported between 2000-2012, with 17 resulting in fatalities (Sabah Wildlife Department 2010A). Despite this, there has been a severe paucity of research carried out on crocodiles in recent decades. Crocodile research in Sabah has been mostly restricted to the west coast, predominantly the Klias River, and has focussed on development of juveniles (Anuar et al. 1996).

Oil palm has become a prolific 'cash-crop' characterised by high yields and fruit value. As of 2012 it was cultivated over 17.1 million ha, mostly in tropical regions. In Sabah, as of 2011, 19.3% of total land area was devoted to the permanent cultivation of oil palm (Abram et al. 2014). Aquatic predators' interactions with oil palm are hitherto unknown; in a time of agricultural and anthropogenic expansion, understanding this interplay could be key to mediating human-crocodile conflict. The fragmented forest-oil palm matrix found in Sabah represents an unexamined habitat to that examined in previous studies. With estuarine crocodiles experiencing large scale recovery in multiple locations throughout their range, this current study has applicability throughout Asia, Australasia, as well as on other large crocodilians throughout the world (Fukuda et al. 2011).

While previous satellite tracking has been centred around establishing base-line patterns of crocodilian movements and ascertaining behavioural traits such as homing in translocated crocodiles (Read et al. 2007), the present study utilised, for the first time in crocodilians, remote download technology. In particular, this approach presented the opportunity for increased tracking duration by reducing battery drain as a limiting factor. The system does, however, require the locating of individuals periodically (weekly or fortnightly) to download the data. This represented a challenge for a far-ranging species such as the estuarine crocodile. The study sought to explore, in more detail, site use and activity patterns, both over the short- and long-term, in estuarine crocodiles.

It is predicted that:

1. High levels of territoriality result in very distinct home ranges, as found in previous studies (Campbell et al. 2013), manifesting as spatial exclusion with individuals holding territories in discrete sections of river.
2. Large males possess the largest territories and these are preferentially found along the main river, allowing greater access to females during the mating season.
3. Individuals are most active at night but remain active during dawn and dusk, providing the greatest risks of human-crocodile conflict with these times coinciding with increased human presence at waterways.

3.2 Methods

3.2.1 *Study Site*

Located on the Kinabatangan River, on the east coast of Sabah, Malaysian Borneo, the Lower Kinabatangan Wildlife Sanctuary (LKWS) (N5.415787; E118.034383) consists of heavily fragmented riparian and semi-inundated forest, dominated by oil palm monocultures with an expanding human presence (Ancrenaz et al. 2004). The lowland semi-inundated forest provides ideal growing conditions for oil palm; it is one of the most heavily converted areas of Sabah in terms of land-use change (Goossens et al. 2005). The region averages 3,000 mm of rainfall per annum, with slight seasonal variation; the wetter months occur from November to May, the drier months from May until October. The general climate is consistent with other tropical regions in that temperature remains fairly constant throughout the year, with close to 100 % humidity (Röper et al. 2013).

As previously described, the Kinabatangan River is the second longest river in Borneo, measuring some 560 km in length; it is also the longest river in Sabah (Boonratana 2000). The study site was positioned more than 150 km from the mouth of the river, outside tidal activity range and ocean salinity. The river harbours a resilient population of estuarine crocodiles (*C. porosus*) that has

seen a significant recovery from an endangered status at the time of its protection in 1982. Capture locations of individuals were spread across approximately 35 km of river length and one oxbow lake (see Fig. 3.1). As well as being geographically convenient, this river stretch was chosen for trapping because of its high abundance of crocodiles compared with other sections of the river (*pers. obs.*).

3.2.2 *Satellite Tag Placement and Tracking*

Satellite tags were placed on crocodiles over the course of four years (2011-2015), all within the LKWS. Satellite tags for placing on adult crocodiles were obtained from two companies: African Wildlife Tracking (AWT – Rietondale, Pretoria, South Africa) and E-obs GmbH (Gruenwald, Germany). African Wildlife Tracking units operated using the Iridium constellation, providing location fixes and allowing for two-way data communication. This enables the researcher to send commands to the tag even when it is already deployed on the individual being monitored. By using geostationary satellites that orbit the Earth at the same rate as the planet rotates on its axis, a spatially-stable system is created that provides 24-hour coverage of an area (Beste 1978). Data are uploaded directly onto a central server, and made secure and accessible to the researcher in both .xls (Excel) and .kml (Google Earth) formats. E-obs tags operate under the Global Positioning System (GPS) constellation. In an attempt to reduce battery consumption, transmission of GPS location data is not possible so the tags must be located and the data retrieved manually using a UHF "yagi" (named after co-inventor Hidetsugu Yagi) directional antenna. Data are transferred in binary format and require the use of a decoder to provide a comma delimited text file, which provides the time-stamped location data.

All tags conformed to scientifically-approved tag:total body weight standards, with tags weighing 400-850 g (<1 % total body weight) (Tuyttens et al. 2002; Theuerkauf et al. 2007). To minimise impact on the crocodile, the tag consisted of reinforced moulded plastic crafted in a streamline shape to reduce drag through the water. The two unit brands were fitted with both satellite transmission hardware and radio transmission capabilities (VHF and UHF). The

VHF component ensured that radio tracking would remain a possibility should there be a loss of satellite signal. As signal detection is unobtainable under more than a few centimetres of water, both VHF and UHF frequencies were transmitted through a 5 cm vertical antenna. This extra elevation enabled data transmission despite shallow submersion of the tag. Each tag was powered by 13,000 mAh of battery power; separating the satellite and VHF hardware power supplies protected against partial tag malfunction. Uniform holes, 7 mm in diameter, ran across the width of the tag for attachment.

Animals were captured using a large steel mesh trap, 12 feet (3.66 m) in length. Comprising of lightweight meshing and a steel frame, the trap was portable and could be moved between different capture locations with a minimal (four person) team. The trigger mechanism, a simple, rope-based system whereby the bait was tied at the back of the trap, used bite tension to release a door latch. Various bait variations were trialled. Chicken intestines were by far the most effective, although pork lungs, hearts and muscle were also used. Successful trapping relied on a combination of bait presentation, scent, quantity and consistency. In general, the most successful baiting preparations were those that had the greatest scent, and were placed just above or barely touching the water's surface.

Once successfully trapped, the captured crocodile was moved to a suitable location where it could with relative ease – and safety – be worked on. At least two jaw ropes were secured before the trap-door was opened; a noose was threaded over the jaws with a stick and a rag placed over the eyes of the animal to reduce both stress and the possibility of aggressive movements from the animal. The two top jaw ropes were held without slack as the door was opened; this maintained an element of control over the animal's movements. At this stage there is a possibility that the captured animal may 'death roll' (a 360° lateral spin that helps when ripping into prey items); this does tire the animal, making subsequent work far easier. Once calmed, the jaws were secured using either rope or strong tape. A capture team (minimum of three persons, with an extra person added for every meter of crocodile over 3 m) was then used to

restrain the crocodile while the jaws were secured; at this stage the blindfold was also repositioned correctly. Larger individuals had their legs tied to prevent further death rolling and other violent movements; morphometric measurements were taken and the animal sexed.

As mentioned (Chapter 2), the tag was placed on the nuchal plate, an area surrounded by six scutes (osteodermal ridges found along the back of crocodilians). The scutes location, as well as the smooth attachment surface provided ideal places for securing the tag. Holes were drilled through the four centrally located scutes and plastic-coated steel wire threaded through the holes and the tag (Kay 2004C). These were then secured at the top of the tag using clamps. Wire was also threaded sub-dermally underneath the nuchal plate (where possible); this secondary attachment is critical to ensure long-term attachment. Each attachment was executed individually ensuring that the integrity of other wires was not compromised in the event of a breakage. In addition to wire–based attachment, the base of the tag was covered with an epoxy resin, providing extra secure attachment. Quick drying resin (5 minute) ensured that by crocodile release the tag was secure.

Prior to release, the captured crocodile was re-positioned to face the river. The legs were untied, ensuring that they remained unable to touch the ground, before the mouth constraint was cut (jaws held tightly shut by hand). Finally, the blindfold was removed triggering instinctive behaviour to enter the water. Once released, tag transmission began immediately. All animals were released without harm and the project had a 0% fatality rate amongst all captured individuals.

3.2.3 *Statistical Analysis*

Home range analyses were conducted in 'R' version 3.0.2. Both minimum convex polygons (MCP) and Kernel utilisation densities (KUD) were constructed to enable comparisons with previous home ranging work. Both analyses were carried-out using packages 'adehabitatHR' and 'PBSmapping'.

Home ranges were constructed using each method providing both total (90%) and core (50%) home ranges.

Local Convex Hull (LoCoH) analyses utilised the packages 'adehabitat', 'rgeos', 'sp', 'gpclib', and 'shapefiles'. Variations of fixed K, fixed R and adaptive (α) LoCoH algorithms were trialled to establish which best represented the data, as well as respect true landscape barriers. Starting values for K, R, and adaptive LoCoHs were estimated following Getz et al. (2007), whereby $k_1=\sqrt{n}$, r_1= half the nearest neighbour distance, and α_1= maximum distance between any two points in a data set. From this starting point, biological factors were taken into account to give a 'best estimate'. Hulls were created by R as shapefiles and imported into ArcGIS 10.0. Isopleth (defining boundaries of a specific area) levels were examined to determine both total (90%) and core (50%) home ranges. In addition, a 99% LoCoH analysis was run to determine range limits. Adaptive (α) LoCoH was deemed the most suitable analysis method based on biological accuracy and boundary plasticity. Values for α were calculated based on straight line distances between the two most outlying points, as well as landscape feasibility of those maps subsequently produced (Getz et al. 2007). LoCoH analyses provided an insight into home range size whilst allowing for spatial exclusion of certain regions that would be automatically included in other analyses (i.e. MCP and kernel analyses). Spatial utilisation does, however, provide a metaphorical one-dimensional picture of how these areas are being occupied; the inclusion of temporal data allows for the identification of key areas of habitat that might otherwise have been overlooked if only spatial data were considered.

t-LoCoH (Time) analyses provided a method of examining the interaction between spatial and temporal variations in home ranges. 'R' packages ('tlocoh', 'sp', 'rgdal', 'rgeos' and 'gpclib') enabled the examination of the impact of time on habitat use; it was possible to examine visitation rates and duration of visits to certain locations. This method introduces the 'S' (Time) variable, which allows for temporal data to be taken into consideration; the value of S indicates the weighting of spatial and temporal factors when creating each individual

hull. Adaptive (α) analysis methods were again employed, giving the isopleths the highest degree of plasticity, observing physical barriers such as riverbanks. α-values were selected by comparing a number of different factors such as barrier observation, GPS location density and "true-hole" observation (where habitat variables ensured that points should not have occurred in an area). S-values were also modified by defining an Inter-Visit Gap (IVG) duration. This length of time ensured that each visit to an isopleth was in fact a discrete occurrence. Using the IVG as the definition of a separate occurrence, the number of visits to a discrete hull (mnlv) and the time spent at the hull during these visits (nsv) were calculated.

3.3 Results

3.3.1 *Crocodile Tagging and Home Range Analysis*

To date, of the 21 crocodiles that have been captured (Table 3.1), 19 have been sampled (morphometrically and genetically); two were released on capture because of their small size. The individuals captured had a mean (±s.e) length of 338.84 (± 90.03) cm (Table 3.1). Of those 19 individuals sampled, 14 were male and five female. Throughout the course of three years of trapping, not a single individual was recaptured despite continued trapping within known individuals' ranges. This indicates a high degree of post-capture trap avoidance. The 21 individuals were captured in 18 discrete locations, along a 35 km stretch of river (Fig. 3.1). Morphometric measurements and tissue samples for genetic analysis were collected from each individual. In addition, each animal was assessed for tagging suitability (with distance between the nuchal scutes being the major factor), 10 individuals were deemed suitable and subsequently tagged (Table 3.2).

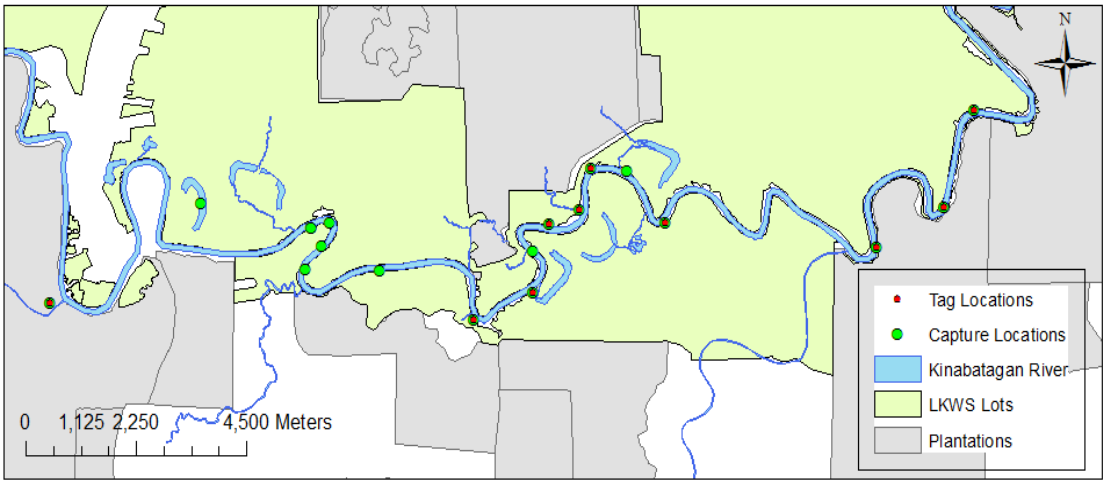


Figure. 3.1 – Discrete capture locations covering a 35 km stretch of the Kinabatangan River. Capture locations denoted in green; locations where individuals tagged indicated by a red point.

Table 3.1. Morphometric measurements (cm) collected for each of the 19 crocodile individual sampled. * denotes individuals (n = 10) that were fitted with a satellite tag. NA denotes information not collected.

ID	Length (cm)	Snout->Vent (cm)	Head (cm)	Tail (cm)	Hind Limb (cm)	Fore Limb (cm)	Head/length ratio	Sex
1*	403	NA	NA	NA	NA	NA	NA	M
2*	356	NA	NA	NA	NA	NA	NA	M
8	272	100	40	50	57	44	6.8	M
9	300	152	42	156	NA	NA	7.14	F
23*	518	234	114	284	119	103	4.54	M
69	332	157	68	181	63	47	4.88	M
70	290	134	61	157	59	44	4.75	M
72*	224	101	42	122	42	31	5.34	F
73*	466	226	85	260	65	84	5.48	M
75	191	91.3	40	100	36	29	4.74	F
76	274	134	57	142	51	39	4.82	M
77	300	151	64	154	56	46	4.66	F
78*	389	177	78	211	69	58	4.98	M
83	375	177	74	203	70	57	5.01	M
92*	396	1823	76	217	76	55	5.19	F
124	278	140	54	140	50	43	5.16	M
142*	313	156	63	160	59	51	4.94	F
143	298	144	60	154	58	48	5.01	F
168*	377	174	75	196	58	57	5.06	M
169*	422	198	80	228	77	63	5.31	M

Satellite units had a mean life span of 71.50 (±53.48) days (Table 3.2); however, two units that failed within several days following release heavily skewed this value. Daily rates of movement (ROMs), or the total average distance moved by an individuals per day, ranged from 1,839.8 m to 6,122.8 m for males, and from 595.8 m to 2,988.5 m for females (Table 3.2).

Table 3.2. Tag performance summary. Tag ID indicates the unique ID used in subsequent analysis. Total length (TL) was measured in cm. Tag longevity was the total life-span of the unit recorded in number of days when there was successful GPS data transmission. Daily rate of movement (ROM md^{-1}) displays the mean daily distance moved by each individual. NA data not retrieved.

Individual ID	Tag ID	TL (cm)	Sex	Tag Longevity (days)	Total Distance Travelled (km)	Daily ROM (md^{-1})
1	M1	403.0	M	53	97.5	1,839.79
2	M2	356.0	M	43	85.4	1,986.60
23	M3	518.0	M	74	236.8	3,200.92
72	F3	224.3	F	5	3.0	595.80
73	M7	466.0	M	2	3.7	1,848.5
78	M4	389.6	M	105	106.3	1,932.49
92	F1	396.0	F	213	636.5	2,988.45
142	F2	313.0	F	26	21.6	829.65
168	M5	377.0	M	33*	194.0	6,122.75
169	M6	422.0	M	20*	NA	NA

Table 3.3. Estimates of home range size using two Local Convex Hull analysis (LoCoH) variations, fixed K and adaptive (α) producing differences in total (90%) and core (50%) home ranges. % core range ratio indicates the percentage of the total home range made up of core areas.

Tag ID	Fixed K – core 50% (km^2)	Adaptive α – core 50% (km^2)	Fixed K – total 90% (km^2)	Adaptive α – total 90% (km^2)	Ratio of core to total
M1	0.016	0.037	0.163	0.150	24.67
M2	0.090	0.077	0.831	0.416	18.51
M3	0.010	0.041	0.370	0.383	10.70
M4	0.017	0.054	0.303	0.310	17.42
M5	0.080	0.120	0.860	0.880	13.64
F1	0.133	0.140	1.666	2.060	6.80
F2	0.010	0.002	0.050	0.019	10.53

Individuals displayed variations in home range size for each of the LoCoH methods utilised. Adaptive LoCoH analyses produced generally larger core home ranges (Table 3.3). Total home ranges (90%) ranged from 0.019 to 2.06 km^2 (Table 3.3); the level of overlap between the core and total home ranges ranged from 6.8% to 24.67%. Males had a larger ratio of core to total home range with a mean (±s.e) of 16.99% (± 5.30%), compared to just 8.66% (± 2.64%) in females (Table 3.3). This indicates that, in general, males were far more site-fidelic and territorial.

Tagged individuals varied in activity levels, with mean weekly movements ranging from 5,392 to 27,710 m, although there were high levels of variability in movement from week to week (Fig. 3.2). Crocodile F1, which had the longest tag longevity of any individual, travelled the longest distance of any tagged individual, cumulating a total distance of 636.5 km over the entire tagging period, however, its daily ROM was only the third highest amongst all seven individuals. Mean (±s.e) daily ROM ranged from 830 m d^{-1} (± 1077 md^{-1}) with F2 and in excess of 6 km travelled by M5. Males had a mean daily ROM of 2,666 m d^{-1} (± 935 md^{-1}) and females 1,909 m d^{-1} (±1,526 md^{-1}).

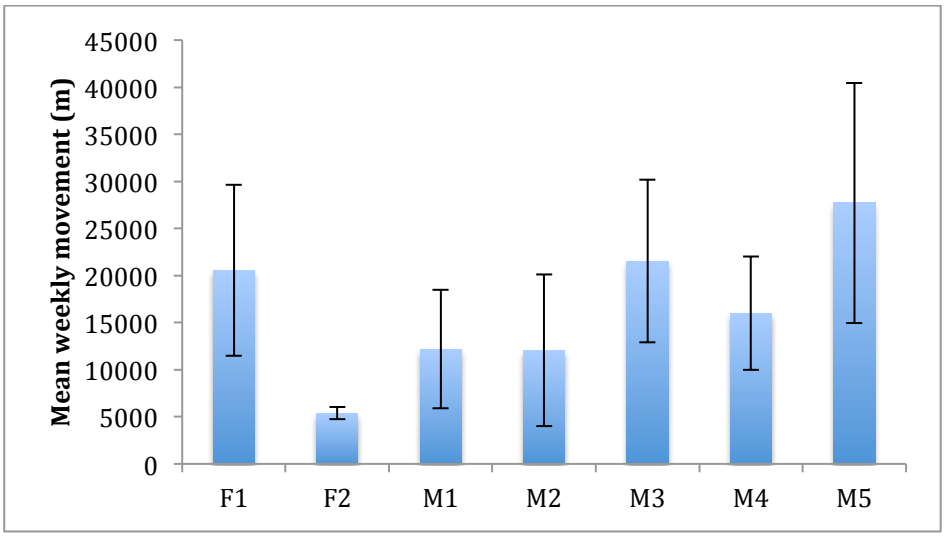


Figure. 3.2 – Mean weekly movement across the total tagged period for each individual (mean values with standard error bars).

Each individual crocodile had higher nocturnal ROMs (Fig. 3.3). The reasoning behind increased rate of movement at night is two-fold, firstly a lack of basking (where an individuals is almost exclusively stationary) and secondly, hunting

behaviours are often associated with increased movements. Decreased movement patterns found throughout diurnal hours found, irrespective of basking suitability, suggests that both factors are contributing to this difference. The mean (±s.e) diurnal (0600-1800 local time (GMT+8)) and nocturnal (1900-0500 local time (GMT+8)) hourly ROMs across all individuals were 121 m h^{-1} (± 20.35 m h^{-1}) and 163 m h^{-1} (± 45.67 m h^{-1}), respectively. A General Linear Model (GLM) to explain distance moved (Gamma GLM with a log-link function) showed that the distance moved was significantly lower by day than by night ($F_{1,5754}$ = 142.9, P < 0.0001), when the significant differences between individuals in overall hourly ROMs were controlled for statistically ($F_{6,5754}$ = 90.6, P < 0.0001).

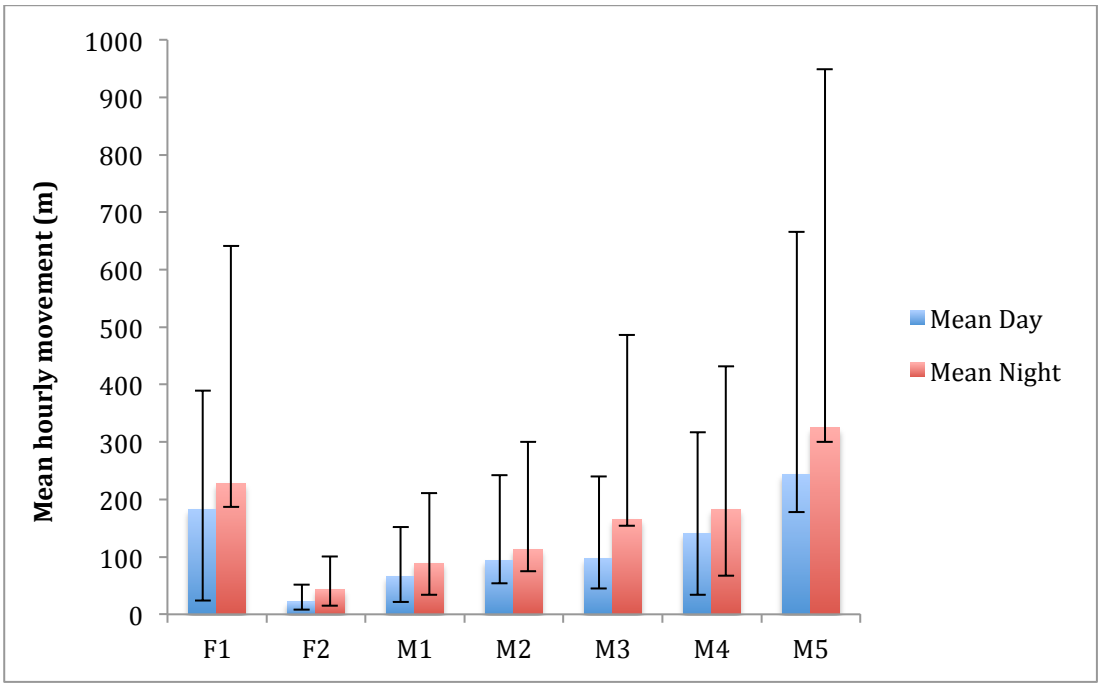


Figure 3.3 – Movements of seven individuals, separating movement rates (mh^{-1}) between diurnal (0600-1800) and nocturnal (1900-0500) hours. Blue bars indicate diurnal movement, and red bars nocturnal movement. Error bars indicate standard error.

Most individuals demonstrated a significant peak in activity at dawn (0600-0700), dusk (1700-1900), or both. A noticeable exception to this pattern was M2 which exhibited a peak in activity between 0900 and 1000 (Fig. 3.4). This peak in activity coincides with increased primate congregations, especially in forested areas (Matsuda et al. 2008). Crocodile M5 had the most erratic

movement patterns; but this may be a consequence of the relatively small amount of data (~6 weeks) collected.

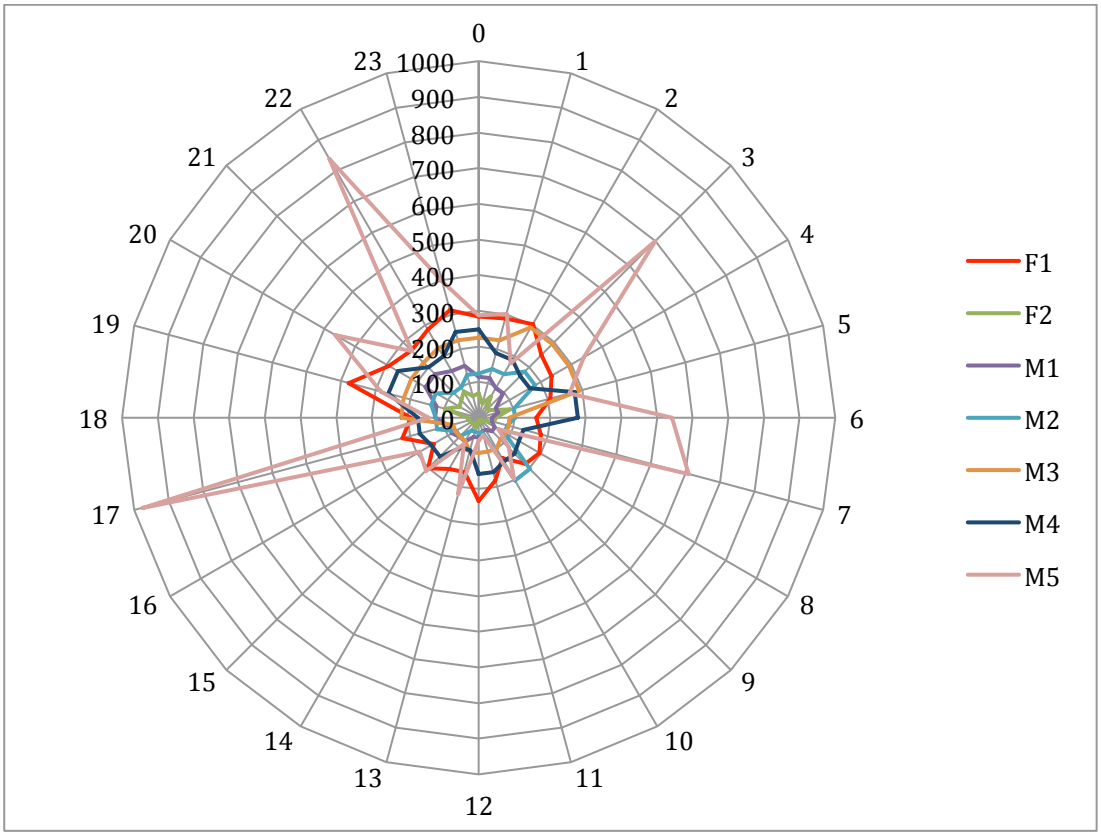


Figure 3.4 – Hourly movement rates (mh^{-1}) for seven crocodiles. This displays different temporal peaks in activity for each of the individuals. Rings display 100 m increments.

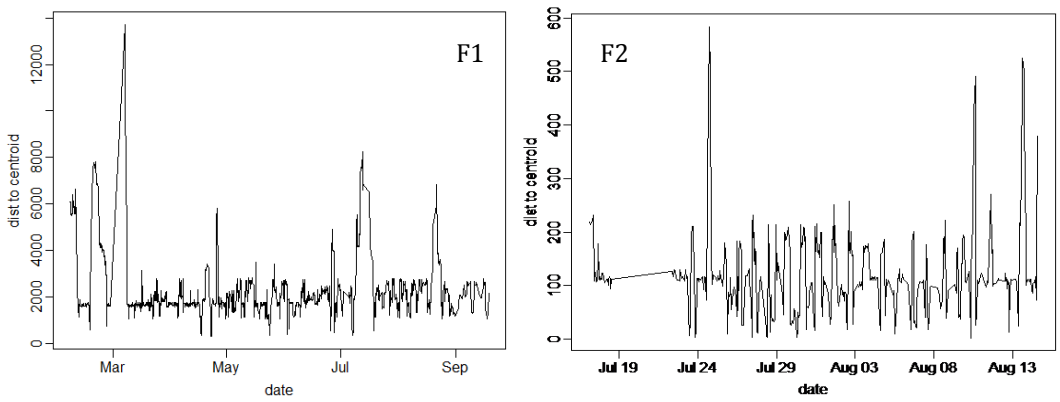
Activity budgets across longer time frames also indicate different strategies between individuals. Despite differences in terms of both movement levels and scales in female crocodiles (Fig. 3.4), their spatial behavioural strategies appear to follow similar trends (Fig. 3.5B). Both F1 and F2 spent large periods of time residing within their core (50%) home range with only brief excursions to areas farther afield. The scale of these excursions was, however, markedly different between the two individuals, with F1 moving more than an order of magnitude further from the core home range than F2.

Males, on the other hand, displayed markedly different strategies, even when comparing individuals of similar size classes (Fig. 3.5B). Both M1 and M3 appeared to follow a similar strategy, exhibiting very regular movements throughout their home ranges; with core home ranges being found close to the

centre of their territories, the frequency and scale of movements are consistent with territorial patrol. Crocodiles M2 and M4 exhibited different strategies, residing on the edges of their territories, each made one noticeable excursion into areas well outside their usual ranges. Exhibiting a third unique strategy type, M5 did not appear to have a true core home range and areas of intense residence were geographically disjunct. This suggests the possible existence of at least two differing strategies within estuarine crocodiles, site-fidelic and nomadic, as described in Campbell et al. (2013).

Both crocodile M2 and M4 made significant excursions upriver to areas well outside of their usual home ranges. Both individuals did, however, halt below a major, busy highway bridge, the only bridge to cross the Kinabatangan, before returning back to their usual home range (Fig. 3.6A). One female, F1, spent the vast majority of her time in the 10 km stretch downriver of this bridge and despite heavy point clustering in the area immediately after the bridge, never passed underneath it despite being less than 20 m from it on multiple occasions (Fig. 3.6B). The other female, F2, was the only individual caught on the other side of the bridge and also made no attempt to pass under the bridge (Fig. 3.6C). The unwillingness to pass this apparent barrier could have both short- and long-term implications for the Kinabatangan population.

A)



B)

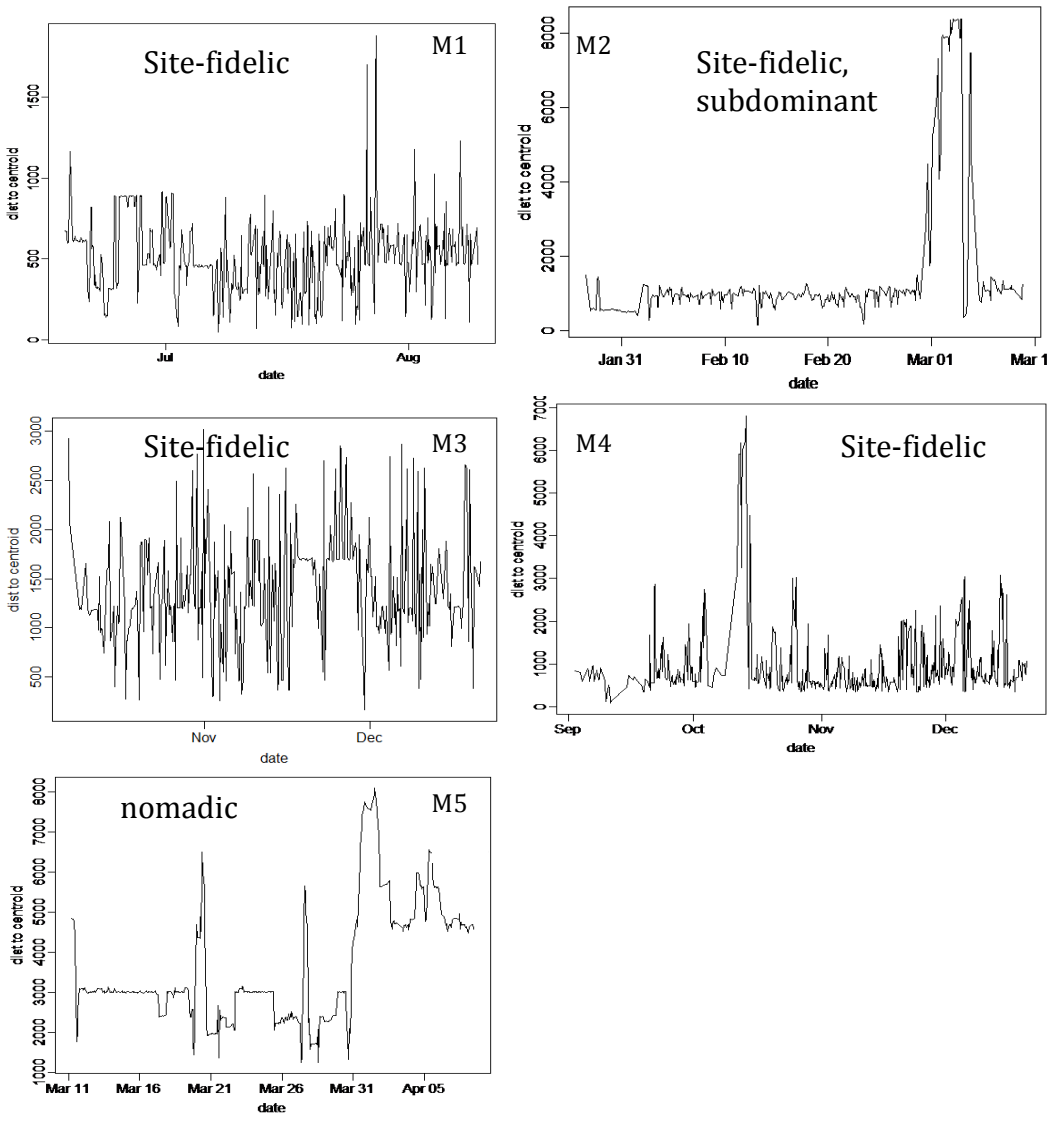


Figure 3.5 – A.) Distance to centroid (m) for females throughout their tagging duration, displaying similar patterns of movement, albeit over different scales and B) Distance to centroid (m) for males throughout their tagging duration, showing a range of movement strategies, varying scales and levels of fidelity to core home ranges.

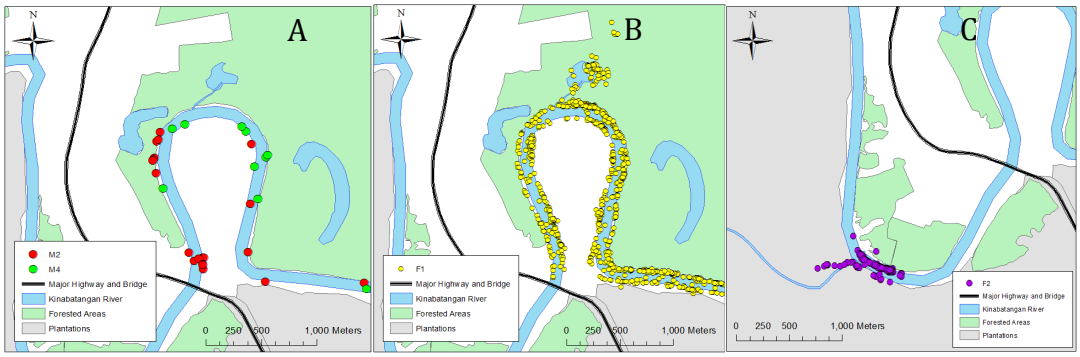


Figure 3.6. Unwillingness by four separate individuals to pass under bridge with heavy traffic. Circles indicate discrete locations of an individual. A) Appears to show bridge restricting attempted dispersal of subordinate males. B) End of range of F1, unclear as to whether this range would have been extended without the presence of the bridge. C) F2's range surrounded by human inhabitants (multiple houses and oil palm) displayed a very restricted range.

Within each individual's home range there were areas that were heavily utilised, as well as areas that were never occupied (Fig 3.7 A-G). Individuals that occupied overlapping territories, for example M1 and M2, exhibited intra-territory avoidance strategies; M2 only rested in areas that were not part of M1's core or total home range when moving between disjointed, geographically isolated sub-portions belonging to its own, non-main river, territory. This appears to show clear territorial behaviour, as well as an established dominance hierarchy. Two males and one female held exclusively main river territories; the two males, measuring 4.03 m and 3.89 m, were, respectively, the third and fourth largest males tagged (Fig. 3.7 A-E). The smallest male, M2 (3.56 m), held an almost exclusively non-main river territory. Two males, M3 (5.18 m) and M5 (3.77 m), held territories including significant areas of both main-river and tributaries. These behaviours suggest that size does play a role in territoriality but that there are other, less obvious factors at work, such as nomadic versus site-fidelic behaviour. Females, by contrast displayed a very different spatial dispersal, with F2 appearing to be constrained by a large anthropogenic presence surrounding the crocodile's home range. No direct correlation between surrounding habitat and home range size was found, with the exception of the formation of barriers presented by human presence (Fig. 3.6).

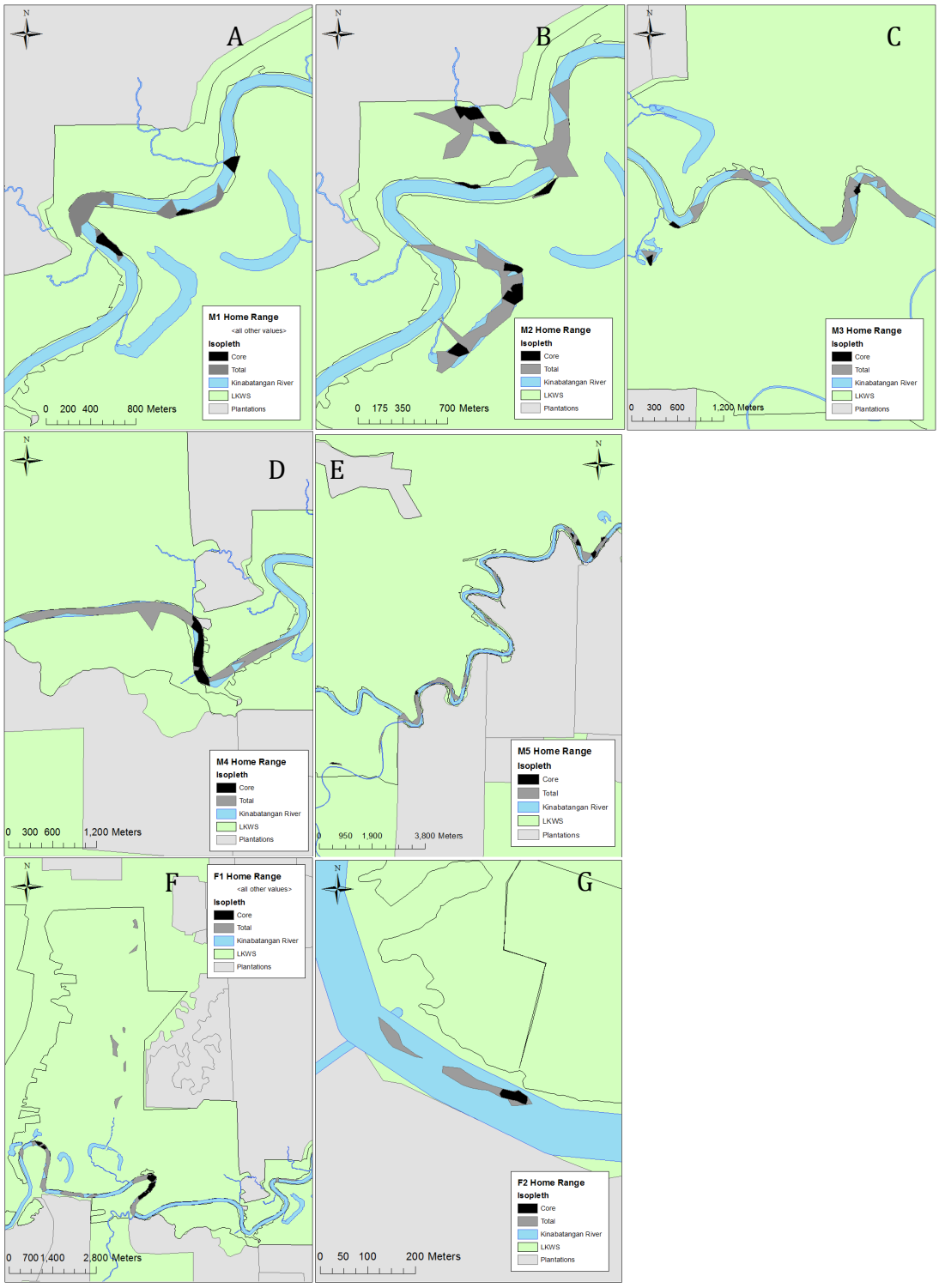


Figure 3.7 - A-E Core (50%) and total (90%) home ranges for crocodiles M1-5. Home ranges computed using Local Convex Hull (LoCoH) analysis. F and G Core (50%) and total (90%) home ranges for F1 and F2. Home ranges computed using Local Convex Hull (LoCoH) analysis. Core home ranges indicated by black isopleths and total home ranges shown in grey.

Analyses of temporal-spatial relationships (t-LoCoH) for each of the individuals displayed marked differences in home range utilisation. Areas deemed to be of

high biological importance were those that included both medium-high levels of visitation and medium-high durations spent in those locations. These locations were indicated by yellow and orange locations throughout (Fig 3.8A). Additional points of interest are those areas with high levels of either visitation (nsv – number of separate visitations to an area) or duration (mnlv – duration spent in a particular area). Across all individuals, areas of high duration (characterised as green hulls (Fig 3.8A and B)) tended to be located either on the outskirts of an individual's (M1, M4, M5, F1 and F2) home range or in areas (M3) that were seldom visited. Areas of concentrated high visitation often represented the core of that individual's home range. The levels of re-visitation between individuals was highly variable, indicating that even within small home ranges certain individuals were far more site-fidelic than others. Crocodile M2, for example, despite occupying a small location, only visited one discrete location more than three times throughout the entirety of his tagging period. This is in stark contrast to M4, who had a similar-sized home range, but up to 19 re-visitations to discrete locations. When examined in terms of re-visitations per day throughout the tagging period, M2 had a revisitation frequency of 0.09 at his most 'favoured' location, compared to 0.18 for M4. With this rate of re-visitation, M4 used seven discrete locations compared to just one for M2.

Durations spent at these discrete locations were also highly variable and seemed to be characteristic of behavioural differences displayed by individuals. F2, whose home range was constrained to a very small stretch of main river spent in excess of 90 hours in a single location. This figure is nine-fold times greater than both M3 and M4, both of whom held substantial main river territories.

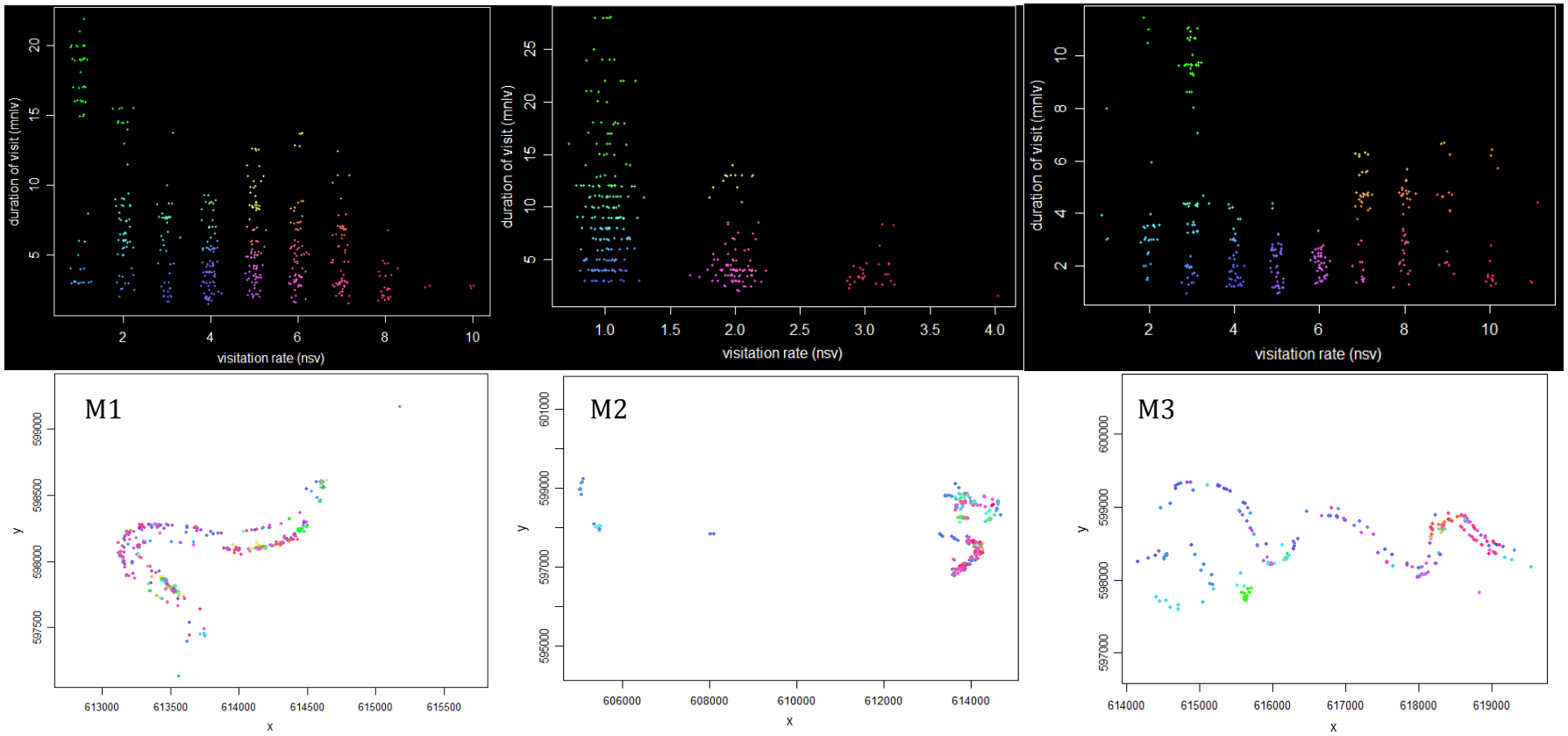


Figure 3.8 A(M1-M3)- Each row of figures display t-LoCoH (fine-scale spatial-temporal analysis) for an individual crocodile. Upper row displays histogram of model results, bottom row the spatial representation of the model. Number of unique visitations to a hull plotted against the amount of time spent (mnlv – hours) in the same hull on the same visit. Visitation was described by any discrete occurrence separated by at least 48 hours. Hulls with high visitation rates and long durations are deemed those with the highest level of biological relevance, providing either biological resources or important territorial landmarks. Points with identical or very similar duration and visitation were "jiggled" to display point density more clearly. Colour ramps are intended to more clearly indicate areas of interest, i.e. locations indicated often for long periods.

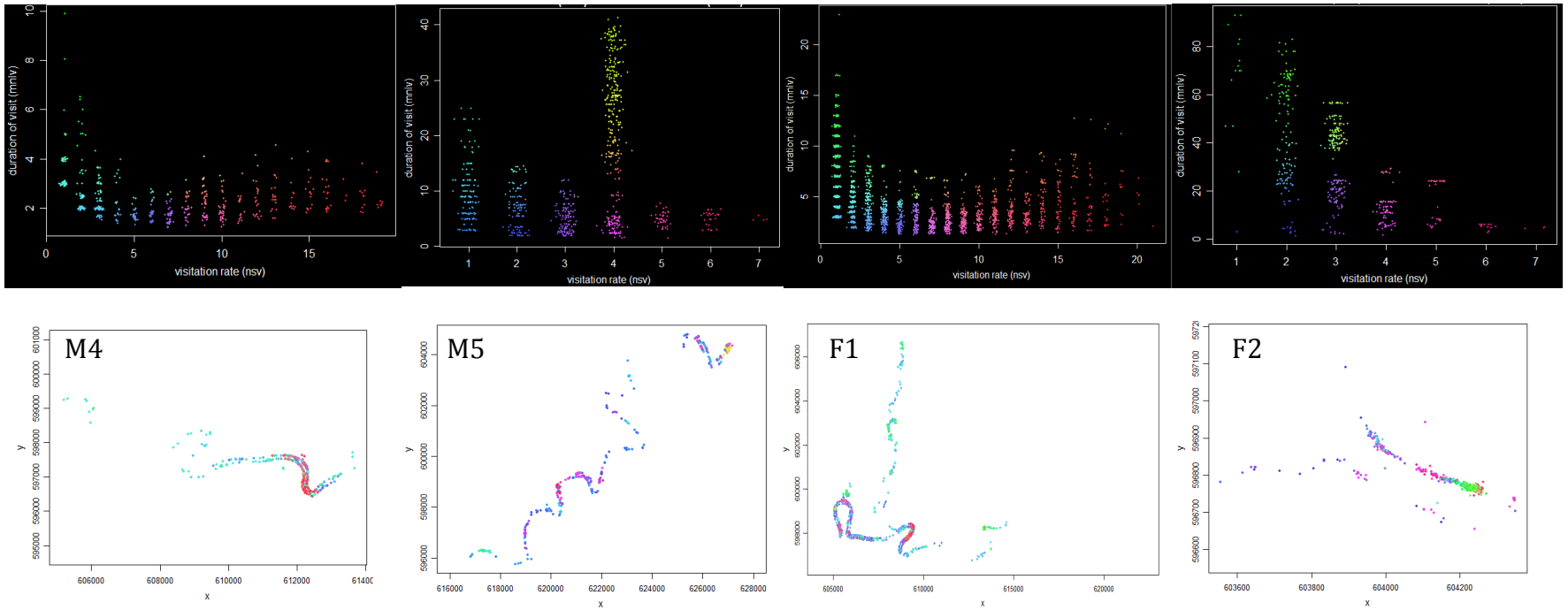


Figure 3.8 B(M4-F2)- Each row of figures display t-LoCoH (fine-scale spatial-temporal analysis) for an individual crocodile. Top row displays histogram of model results, bottom row to the spatial representation of the model. Number of unique visitations to a hull plotted against the amount of time spent (mnlv – hours) in the same hull on the same visit. Visitation was described by any discrete occurrence separated by at least 48 hours. Hulls with high visitation rates and long durations are deemed those with the highest level of biological relevance, providing either biological resources or important territorial landmarks. Points with identical or very similar duration and visitation were "jiggled" to display point density more clearly. Colour ramps are intended to more clearly indicate areas of interest, i.e. locations indicated often for long periods.

3.4 Discussion

This study represents an initial insight into the movement patterns of crocodilians in Borneo. The rapidly changing environment in Sabah, and the fragmented habitat found in the LKWS, potentially provides an applicable model for forest ecosystems worldwide, shedding light on behavioural responses of crocodiles to human-modified landscapes.

The LKWS's strong and stable crocodile population consists of a large density of breeding sized adults; this is evident from the number of individuals captured along a relatively short stretch of the river. Crocodiles were only ever captured on one occasion, with not a single recapture of any trap-caught individual throughout the study period (2011-2015). This was despite multiple trapping events in the same locations, and within the known home ranges of several previously caught crocodiles. This suggests that crocodile individuals in the Kinabatangan become extremely trap-shy once captured. This wariness of humans could be one of the reasons that the river still harbours relatively large numbers of adults, with body length in excess of 5 m, despite the heavy hunting activity that occurred prior to the 1980s. Several of the crocodiles that were captured in the current study exhibited potential human-conflict wounds; these ranged from potential gunshot wounds to propeller injuries.

Individuals' movements varied greatly, displaying similar male behavioural strategies to those described by Campbell et al. (2013), with males being of either a site-fidelic (M1-4) or nomadic (M5) 'type'. These authors, however, reported far lower ROMs for females than found in the current study which suggests that females do employ differing behavioural strategies, with one female (F2) behaving in a similar fashion to previous studies, but F1 employing a distinctive nomadic strategy. The daily ROMs of males had a mean (±s.e) of 2.66 kmd^{-1} (± 1.01 kmd^{-1}) per day, this movement being larger than the mean territory size of the individuals studied. This level of movement within a territory suggests that there is significant patrolling of

territorial borders, and this is likely to be as a deterrent to encroachment by other males. This supports the hypothesis that individuals are defending discrete sections of river, however, there is inconclusive evidence with regards to the differences in ROM between males and females. The limitations of sample size mean that these data should be treated as a preliminary look at crocodilian behaviour in the Kinabatangan, however with large frequencies of data points collected for each individual the study provides a step forward in behavioural understanding.

All individuals were more active at night than during the day, and most exhibited peak activity at dawn, dusk, or both, this supports Hypothesis 4. This movement pattern could be explained by the initiation and cessation of hunting or scavenging behaviours most associated with nocturnal predators (Martin, 2007). Times of increased activity could bring the crocodiles into more frequent human contact; given the motive behind this increased activity, the initiation of hunting behaviour, coupled with the large number of people who congregate at the water in the early evening, the period 1700-1900 represents the time with the greatest risk of human attack.

Examining the distance to centroids of each of the individual's compounds the concept that different behavioural strategies are found within crocodilian populations, and that those behavioural traits are not necessarily linked to the overall length of an individual. Crocodile M5, the only nomadic male monitored during the study, was the fourth largest of the seven males tagged in the study. This suggests that the trigger for nomadic behaviour may be more closely linked to an individual's "neighbours", and how large and aggressive the surrounding males are. Crocodile M5, for example, bordered the territory of M3, the largest of all of the individuals, and seemed to share a spatial overlap in home range with M7, the most aggressive individual in the area. This crocodile is known to have challenged and even attempted to flip fishing boats (*pers. comm*). Despite the presence of nomadic behaviour, this present study highlights the frequency of highly territorial behaviour that not only formed spatially exclusive sections of

main river, as predicted, but also resulted in isolation to flooded forest and oxbow lakes (M2). This suggests that, in line with Hypothesis 3, larger males are preferentially selecting stretches of main river as territories.

Four of the five males occupied distinct home ranges. Of these, the largest three all held main river territories that were both spatially- and temporally-exclusive. The indication that main river territories provide an evolutionary advantage is compounded by the fact that one (F1) of the two females tagged passed through each of the territories of those males holding a main river territory. This appears to contradict the findings of Kay (2004A) who showed males occupying ranges for a period of days or weeks, before moving on to find a new home range. This could be a result of barriers preventing movements and dispersal of individuals. Whilst it was found that the main river clearly represented desirable territories, the size of these territories seemed not to be affected by the presence of either forest or oil palm plantation bank habitat. Instead it seemed that the presence of human activity itself was the driving factor behind territory size, location and allocation.

Despite not providing a physical barrier, the large bridge located at the local village (Fig. 3.6) appeared to provide an impassable barrier to four individuals, three tagged downriver and one upriver. There are two potential causes of this reluctance to cross: firstly, as the only bridge crossing the entire Kinabatangan River, there is a heavy continuous traffic load with a large number of trucks. This activity produces considerable volumes of noise, possibly passed through concrete columns underneath the water. Secondly, the bridge is situated at a local village with more than 20 boats moored under the bridge itself. While human activity is mostly diurnal, the presence of lights and village-associated noise are perennial. Most likely, combinations of these two factors are preventing any individual from dispersing either up- or downriver (depending on tagging site) from their current home ranges. It is also worth noting that the only individuals found on the ‘up-river’ side of the bridge had a highly restricted home range

surrounded by houses and high levels of boat traffic. This type of anthropogenic barrier has the potential to increase conflict situations as growing populations are prevented from dispersing, causing overcrowding and forcing individuals into sub-optimal territories. The majority of barriers identified in other crocodilian studies, for example bars and rapids, are those that cause a physical impediment to movement and dispersal activity (Kay 2004; Letnic & Connors 2006; Nair et al. 2012). The discovery of non-physical barriers has important implications for crocodile management, not only in terms of the genetic health of a population, but also when planning and implementing crocodile exclusion zones.

The Kinabatangan River, and in particular the study region, is heavily utilised by fishermen who regularly net across the entire river (*pers. obs.*); this activity does not appear to limit crocodile movement. Crocodile killings perpetrated by humans appear to be rare in the area with only one death directly linked to fishing activities noted during the study period. In this instance, a breeding age female was found drowned after becoming entangled in a fishing net (*pers. obs.*). During 2015, however, at least three crocodile carcasses have been found with potential 'bullet wounds' (*pers. comm* Sabah Wildlife Department); this suggests that with rising attack numbers that conflict is becoming a far more decisive issue.

t-LoCoH analyses provided a new perspective on crocodilian movement patterns. The indication of temporal home range utilisation is a novel finding. With each of the individuals assessed, areas of high biological importance consisted of core home ranges. This shows that only a fraction of any given home range is heavily utilised and that individuals tend to select areas preferentially. Individuals spent considerable time in these areas, and also returned to the area many times during the tagging period. When looking at visitation and duration of stays individually, areas of high visitation were generally found throughout the home range, however, the levels of re-visitation were indicative of differing behavioural strategies displayed in both males and females. The fact that many of the crocodiles re-

visited a large number of discrete locations throughout their entire home range is indicative of home range patrolling and territoriality. Areas of high duration were often found in areas spatially separated from the majority of an individual's home range, providing the animal with time to recover from swimming distances they were not accustomed to.

This study represents a 'first look' at how fragmented habitats are affecting movement and home ranging strategies of adult crocodilians. The fact that direct human disturbance appears to be having the greatest impact on ranging and dispersal behaviours implies active avoidance on the part of the crocodiles. This has wide reaching repercussions when considering human-crocodile conflict and suggests that there is the potential for an escalation of the situation should further anthropogenic expansion further divide and restrict growing crocodile populations. It is worth noting that the Kinabatangan still retains relatively large quantities of forest compared to other oil palm dominated rivers such as those found in northern Sabah. A lack of attack reporting, especially in oil palm dominated regions, may be masking a situation far wore than is currently recognised. Crocodiles are hugely resilient organisms having survived may changes in both climate and landscape, however, the rate of anthropogenic expansion currently happening worldwide has thrust species into a conflict that can potentially be highly deleterious for this important aquatic predator.

Chapter 4 – Use of Drone Technology as a Tool for Behavioural Research: a case study of crocodilian nesting

Adaptation of an article published in Herpetological Conservation and Biology.*

4.1 Introduction

Accurate behavioural monitoring of any species is crucial in the drafting of conservation and management plans (Kleiman et al. 1986). To obtain such data, a wide array of methodologies have been conceived and implemented, each associated with its own set of intrinsic limitations. Direct observations, while effective, may only capture a fraction of behaviours with an associated ongoing risk of behavioural modification through human presence (Bejder et al. 2009). The use of remote, non-invasive, behavioural monitoring provides the key to observing natural behaviours. Technologies, such as camera trapping, can provide insights into some natural behaviour, but are restricted to discrete locations. The ability to track animals remotely and continuously with Global Positioning System (GPS) technologies has led to major advances in studies of wildlife behaviour and habitat usage, while also providing more accurate home range estimates (Seegar et al. 1996; Hebblewhite & Haydon 2010). Despite these advances, the monitoring of more cryptic, fine scale behaviours remains a challenge.

The use of unmanned aerial vehicles (UAVs), or "drones", is a burgeoning facet of conservation biology with the potential to revolutionize the way in which animals and habitats are monitored. Drone technology in itself is not a new development, with military applications having expanded rapidly over the past decade (Vogel 2010). These tools have, however, always been too expensive for scientific application. The development of low-cost, open-sourced alternatives has brought the technology within financial reach of researchers and conservationists; low-cost aircraft can be purchased and

*Evans, L. J., Jones, T. H., Pang, K., Evans, M. N., Saimin, S., & Goossens, B. (2015). Use of drone technology as a tool for behavioral research: a case study of crocodilian nesting. *Herpetological Conservation and Biology, 10*(1), 90–98.

equipped for as little as £1,500 (~$2,000 US) (Koh & Wich 2012). The open-sourced nature of the technology, coupled with the large online community of hobbyists, as well as professionals, could lead to drone use being a viable staple of conservation biology.

One example of a taxon whose biology and ecology can be much better investigated and understood by applying drone technology is crocodilians. This is particularly so in the detection and mapping of crocodile nesting behaviour. Until recently, crocodilian nest studies focusing on location and distribution have used helicopter and airboat surveys both for identification and validation of nest sites (Magnusson et al. 1978; Rice et al. 2000; Harvey & Hill 2003). These techniques are financially costly, despite being more feasible for more remote regions. Walked surveys have, until now, often been used in conjunction with helicopter surveys to find and validate crocodile nesting habits (Harvey & Hill 2003).

Estuarine crocodiles, *Crocodylus porosus*, are the largest extant crocodilians, with individuals reaching in excess of 6 m in length (Britton et al. 2012). Nesting behaviour in *C. porosus* involves females building mounds of both vegetation and mud (Webb et al. 1977). Nests are usually built within 5–10 m of permanent water sources, however, they can be as much as 100 m from deep water (Webb et al. 1977; Harvey & Hill 2003). Work on *C. porosus* nesting has been largely confined to Australia, with a paucity of studies in South East Asia.

As well as being highly labour-intensive, there are numerous challenges involved in the surveying of *C. porosus* nests in mixed tropical habitats. Semi-inundated and swamp forests, with tall grass, pose logistical challenges to nest detection efforts, as well as being potentially dangerous obstacles for surveyors. Drone technology provides a logical next step in nesting monitoring methodology, due to relatively low initial cost, repeatability, and flexibility. In this study, the potential use of such

applications is described on *C. porosus* in the Lower Kinabatangan Wildlife Sanctuary (LKWS), Sabah, Malaysia.

4.2 Materials and Methods

Flights were carried out using a fixed-wing drone (Bormatec-MAJA: Bormatec, Mooswiesen, Ravensburg, Germany), with a wingspan of 1.8 m and a weight of 3 kg, comprised largely of expanded polypropylene. The aircraft was capable of bearing a payload of about 500 g. This allowed the addition of camera equipment (250 g), as well as maximizing the flight time on a single battery (3S 4000 mAh). The aircraft was able to make single flights of 23–25 km, or approximately 250 ha, if flown in grid formation, with flights of this distance taking between 30 and 40 min to complete (Fig. 4.1). This range could have been extended to a maximum of 50-70 km with the use of larger 3S 10000 mAh batteries, but this would have resulted in a reduction in payload capability.

Camera choice (Model S100, Canon, Ota, Tokyo, Japan) for use during flights was based on both cost and performance capabilities, the unit was customised with a firmware (a set of instructions stored in ROM Read Only Memory) enhancement created using a Canon Hack Development Kit (CHDK), which is stored in a camera memory card. A special inter-volameter script was developed by Conservation Drones (www.conservationdrones.org) allowing the camera to take pictures at regular intervals. To provide sufficient picture overlap of about 50%, shutter intervals were calculated by evaluating drone airspeed and altitude (Koh and Wich 2012).

Flights were planned and uploaded through the Auto Pilot Module APM mission planner, an open sourced ground control station software, in concordance with methods described by Koh and Wich (Fig. 4.1). The transects flown were 170 m apart, this distance was based on the altitude and the resolution of the camera. As the flights were flown at 300 m, the

firmware was instructed to take a single picture every 3 sec. This resulted in a picture overlap in excess of 60%.

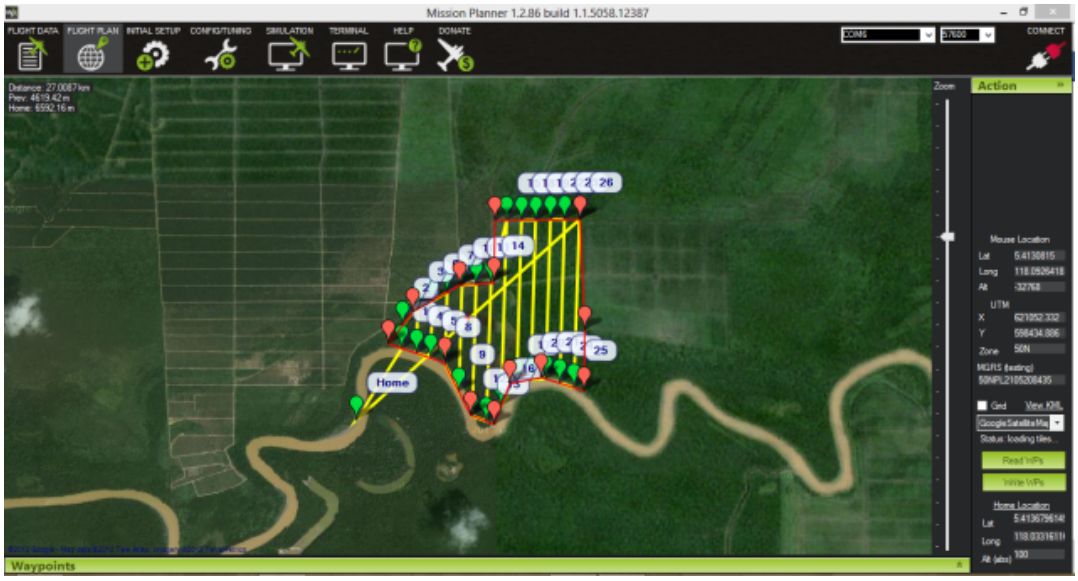


Figure 4.1. Planning missions using Auto Pilot Module (APM) planning software. Flights were flown in a grid formation with transects separated by a predetermined distance to allow sufficient overlap for stitching (joining of images together into composite). Transect separation was 170 m. The image displays the actual route taken by the unmanned aerial vehicle (UAV) during a single flight covering around 300 ha.

The drone was launched manually and it flew via remote control until reaching an altitude of about 100 m. Once this height was reached, auto-pilot was engaged and controlled via the mission planner, which ordered the drone to follow predefined coordinates at the cruising altitude. Average drone airspeeds in a range between 40–50 kmph were recorded, but this was heavily influenced by wind speed and direction.

Flying at 300 m allowed maximum landscape coverage, as well as ensuring good clearance above all tree lines. Also, at this altitude, picture resolution averaged 8–9 cm per pixel. This allowed easy detection of any potential crocodile nests, which measure 1–2 m in diameter (Webb et al. 1977). Greater resolutions of pictures could be achieved, but to attain this would require flying at a lower altitude. When flown at 200 m, flights yielded a resolution of around 5–6 cm per pixel. This resulted in a reduction in aerial

coverage per flight of around 26%, given the same payload and battery capabilities. This method can be used, however, when images are not sufficiently clear to identify nesting mounds effectively.

Missions were piloted between 14 and 18 October 2013, Sabah's wet season, and during the nesting season for the region's crocodiles. Flights were flown in four key, predetermined areas based on direct observations of both crocodile juvenile prevalence and suitable habitat. The survey areas covered two of the largest tributaries along the Kinabatangan. Both tributaries are sites of human fatalities from crocodile attacks within the last two years and are subject to direct encroachment from agriculture. Two additional areas were selected as they were characterized by large areas of swamp-land and drying, old oxbow lakes, both excellent crocodile nesting habitat. Flight missions were timed to occur at different times to establish which daylight conditions resulted in the best nest detection and image stitching capability. Flights were conducted in early morning (0700–0900), late morning (0900–1100), or afternoon (1400–1600). Once retrieved, images were stitched together using program Pix4D (1015 Lausanne, Switzerland). The minimum system requirements for this program include an i7 quad core processor along with 32 GB RAM Random Access Memory and an SSD Solid State hard drive.

The study site consisted of a stretch of the LKWS encompassing Lots 5 (N5.423742°, E118.055597°), 6 (N 5.397137°, E 118.073509°) and 7 (N 5.414195°, E 117.972°; Fig. 4.2). This region is comprised of a forest-oil palm matrix, located some 150 km from the Sulu Sea in an area with a large and growing crocodile population (Luke Evans, *unpub. data*). The nesting of crocodiles in the area has not been studied in detail, although several unsuccessful helicopter surveys have been conducted within the Kinabatangan (Jibius Dausip, *pers. comm*). The use of drone technology allowed for the specific targeting of areas deemed suitable for assessment without the need to fly over vast tracks of unsuitable habitat.

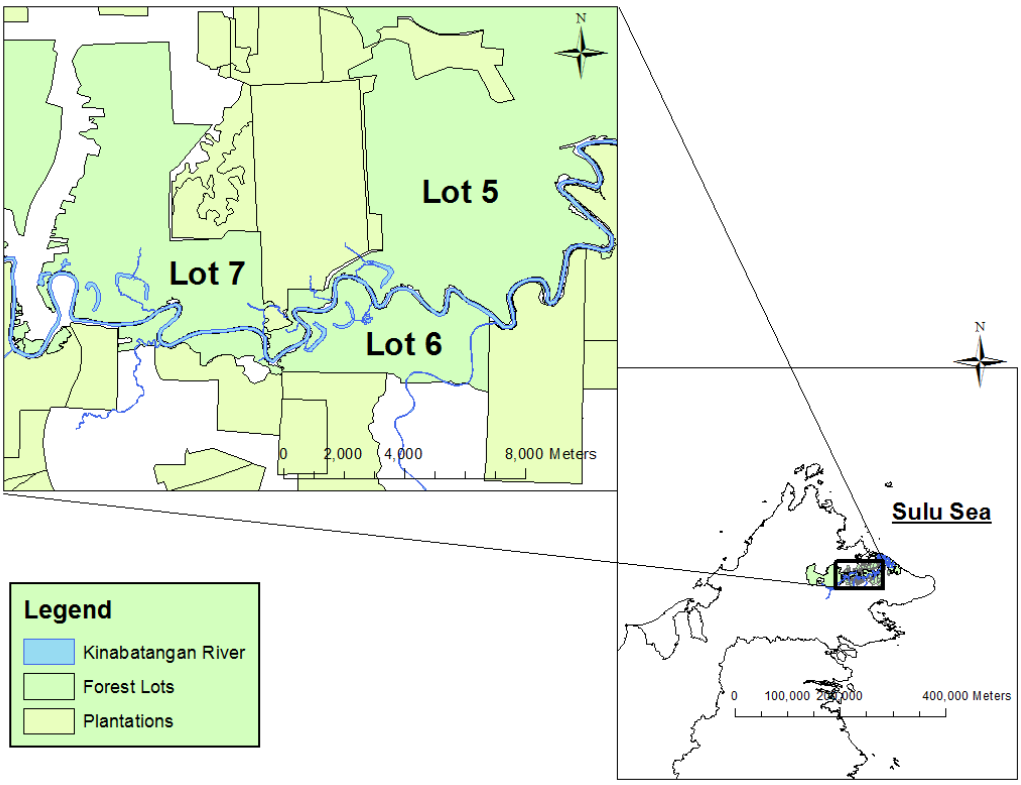


Figure 4.2. The study site situated in the Lower Kinabatangan Wildlife Sanctuary (LKWS), located within the Malaysian state of Sabah. The survey area comprised parts of Lots 5, 6, and 7

4.3 Results

Five preliminary flights were conducted, assessing areas ranging between 200 and 390 ha in area, resulting in a total survey area of 1,550 ha. Three missions were flown at either 0700–0900 or 0900–1100, and an additional two at 1400–1600. Images captured while flying in the 0700–0900 period provided the clearest stitched collages (Fig. 4.3A), whereas images flown 1000–1500 were more fragmented during the stitching process (Fig. 4.3B).

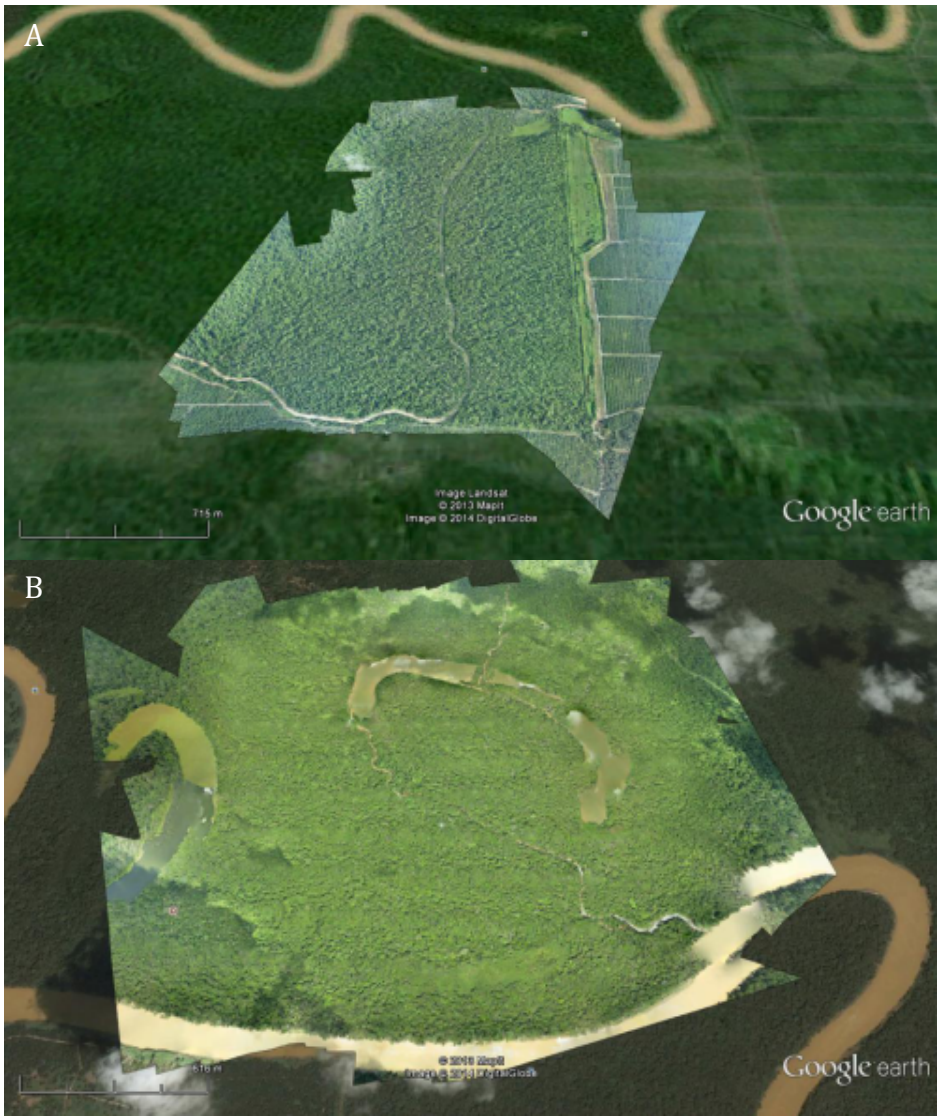


Figure 4.3. A) Stitched image of 280 ha fight flown at 0700 local time GMT+8, where the stitching quality was of high. B) Stitched image of 390 ha flight flown at 1400 local time GMT +8, where the stitching quality was of low.

Nests were identified by searching stitched images and zooming in on specific areas of interest identified from the original images (Figs. 4.4A and 4.4B). Following the flight missions, three potential nests were identified. All potential nests were located in the drying remnants of old oxbow lakes. Following detection, we sought to validate the nest site on foot, with care taken to ensure no female presence (Fig. 4.4C). Of these three, two were confirmed as active nest sites. Both were situated in seasonally flooded areas and were within 150 m of a permanent waterway. One confirmed nest was located just 150 m from a large plantation, within audible range of the

plantation. The third, potential, nest was located but found to be an area of dead grass.

Figure 4.4 A) The raw aerial image of a potential nest (red outline) located in Koyah tributary, one of the largest tributaries of the Kinabatangan River. B) Zoomed image displaying potential nest. C) Confirmed nest found in Koyah tributary. The central mound is surrounded by marshy wallows used by a female. The mound measured 60 cm in height and 1.5 m in diameter.

4.4 Discussion

The study confirms that drone technology can be applied to surveys of crocodile nesting. Given this, drone surveys have the potential to become the standard mechanism for crocodile nest detection. The presence of crocodile nests in areas of low to medium levels of human disturbance were

confirmed, with one of the two confirmed nests being identified in close proximity to an oil palm plantation. The study did not set out to assess detection rates. The financial benefits associated with this approach coupled with the ease of repeatability can ensure enhanced understanding of crocodile nesting ecology and lead to long-term monitoring efforts, even in remote locations. Beyond this, the technology could also have considerable impact within the field of behavioural ecology. It must, however, be emphasized that scientific applications of drone technology are still in their infancy. Further improvements in flight time and duration over the coming decade will ensure that many other applications become feasible. During this study, for example, it was possible to detect a recent (about one month old) 5.51 ha forest clearance situated directly adjacent to the LKWS boundary. While legal, such encroaching conversions need monitoring to ensure wildlife sanctuary boundaries are not breached and to maintain remaining forest cover in an already highly fragmented region. This type of monitoring has the potential to alter completely the way in which protected areas are managed, with real-time boundary security a true barrier to illegal logging. Close monitoring such as this can also aid in the understanding of habitat requirements of many species, thus enabling better resource management to sustain the remaining fauna of the region.

One confirmed nest, located roughly 150 m from the edge of a large plantation, was found in a strip of forest currently outside the LKWS boundaries. This, coupled with its close proximity to an existing plantation, makes the area highly susceptible to habitat conversion. The discovery of its use as active crocodile nesting grounds could lead to its subsequent protection. Gaining a change in land protection status can be incredibly challenging; however, the presence of important nesting grounds of any protected species can provide the impetus needed to convince authorities that the status change is warranted.

Increases in drone reliability, flight speed, and duration have diversified the applicability of UAVs. The creation of real-time landscape maps, the

detection of encroachment and breaching of protected areas, as well as aerial anti-poaching patrols have all become possible as a direct consequence of recent advances in battery capabilities and motor performance. With developing anti-poaching approaches a topic of major conservation importance, the potential of drone technology to contribute to both the monitoring and protection of both highly endangered animals and their habitat is a major possibility.

The ability of drone technology to aid in multiple aspects of both applied conservation and active research have led to its burgeoning prominence in 21st Century science (Estrada & Butler 2012; Koh & Wich 2012). This trend is likely to continue with rapidly improving hardware and firmware. Despite this rapid improvement, many applications do, however, remain unfeasible given current technological limitations. Factors such as component weight versus output is one area that could increase both flight duration and airspeed. Additionally, overall reliability of the major components needs to be improved to give the operator confidence when flying long-range missions. Finally, these improvements must not result in price increases that take the system beyond the financial reach of research and conservation organizations. Future research should focus on quantifying nest detection probability during drone surveys, this will enhance its use both for research and management purposes. This is one of the aims of Chapter 5 and is addressed therein.

Chapter 5 – Spatial Ecology of *Crocodylus porosus* Nesting in a Fragmented Habitat.

5.1 Introduction

Crocodiles are a cryptic guild of animals; it is probably this, their historic association with human settlements (e.g. cave paintings, Mayan drawings (Werness 2006)), as well as their secretive and poorly-understood life histories that has made them the 'stuff' of many myths and legends (Brazaitis et al. 2009; Caldicott et al. 2005). Many tribal people hold them in high esteem; belief in the spiritual, and even supernatural, powers of crocodiles are particularly prevalent with the Iban people of northern Borneo. They hold that no crocodile should be killed unless human attacks have occurred (Stuebing 1983). As a consequence of these self-imposed guidelines, there remain a large number of 'exceptionally'-sized crocodilian (greater than 6 m in length) individuals throughout Sabah and Sarawak (*pers. obs*.).

Exposing cryptic animal behaviours has long been a challenge for zoologists, particularly for those species that reside in remote areas. Cryptic nesting behaviours are not found exclusively in crocodilians; they are exhibited by a wide array of large reptiles including, for example, the yellow-spotted monitor (*Varanus panoptes*) and the striped mud turtle (*Kinosternon baurii*) (Doody et al. 2014). These animals cryptically nest using a wide variety of media and a broad range of strategies. Surveys of reptile nesting have historically aided in the production of baseline population figures, as well as facilitating the detection of population declines (Hilderbrand (1982) in Gibbon et al. (2000)). In fact, such assessments have provided "early warnings" of catastrophic population crashes allowing the formulation of effective management schemes both to prevent future crashes and to map population recoveries (e.g. Balazs & Chaloupka 2004; Schroeder & Murphy 1999). The effectiveness of such monitoring studies is, however, reliant on the observer's ability to detect a high proportion of the total number of nests

located in a given area. This is one of many weaknesses associated with the approach and has resulted in numerous inaccurate reptile population estimates. Typically, the consequence of this is an underestimation of the challenges facing global reptile populations (Gibbon et al. 2000). It is undeniable that the development of more suitable and stringent nesting surveys is a high-priority conservation tool requirement for the entire class of Reptilia.

The history of crocodilian nesting studies began in the 1960s (Joanen 1964; Pooley 1969); detailed work on nesting behaviour across species did not, however, commence until the 1970s. *Crocodylus porosus* nesting was first examined in detail by Webb et al. (1977). This early work focussed on the mechanics of nesting behaviour (for example, what building materials were used), and recording nest characteristics such as temperature as well as the number of eggs oviposited. Spatial analyses, or assessments of the distribution of nests in a given area, have not been the focus of many studies to date. Possibly the best example is work carried out by Harvey and Hill (2003). Using Landsat™ image analysis (see below) to classify areas by vegetation types, these workers used a Boolean overlay approach to determine potentially suitable habitat. Historically, there have been a number of issues that limit the effectiveness of nesting studies, particularly when attempting to calculate figures of nest density in a given area. Firstly, it is very difficult to determine that all nests in a region have been discovered; this means that nest density calculations are always rough approximations and usually under-estimated. Secondly, studies of this nature are often prohibitively expensive, relying on the use of either helicopters and airboats, or time-costly walked surveys.

In Chapter 4, the feasibility and potential applicability of drone technology as a tool for crocodilian nesting research was explored. In its commercial infancy, and although becoming more affordable, the costs are still beyond the means of many small monitoring programmes. The traditional form of aerial photography that has been utilised in active research, especially

focussing on land-use change, is Landsat™ imagery (Seto et al. 2002; Shalaby & Tateishi 2007). The medium-level resolution photography provided by this approach is sufficient to identify broad habitat features (for example, to differentiate forest from oil palm plantation), but lacks the resolution necessary for detection of features measuring less than some 30 m in diameter (Yang et al. 2003). Drone technology does provide a means to increase the resolution of aerial photography, without wildly spiralling costs.

The use of drone technology in conservation and biological management programmes is a relatively recent development (Everaerts 2008; Koh & Wich 2012). Over the past decade, drones have been employed to service a host of ecological needs. Applications such as the monitoring of Eurasian beaver reintroductions (Puttock et al. 2015), surveying tree falls (Inoue et al. 2014), determination of forest gaps (Getzin et al. 2014), mosquito pest management (Amenyo et al. 2014) and nesting of canopy birds (Weissensteiner et al. 2015) are just a few of the research fields in which the technology has been applied. As well as for research, drones are also used in active conservation practise; for example, in the monitoring of poaching activities perpetrated against both the black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses (Mulero-Pázmány et al. 2014). The flexibility of the technology to perform in remote and urban locations alike means that, as long as weather conditions allow, drones can be a financially accessible method of surveying most areas on Earth. One challenge encountered when using traditional aerial photography is that some areas are obscured from view; this is particularly problematic in forested areas. Infra-red cameras have been employed to circumvent this issue, particularly when performing counts of individuals of a species (Mulero-Pázmány et al. 2014). These thermal cameras are, however, more than tenfold the price of traditionally-used visible-spectrum stills cameras. The use of drones in crocodile nesting could demonstrate the feasibility and applicability of the technology to other facets of crocodilian research. Martin et al. (2012) demonstrated that adult alligators could be easily identified aerially using

drone technology, suggesting that count surveys and future population density estimates could, at least in part, be calculated by such surveys.

Selective land conversion for agricultural purposes raises a number of potential issues for crocodile nesting. Oil palm (*Elaeis guineensis*) plantations are prevalent throughout tropical regions and are expanding rapidly into previously remote areas (Abram et al. 2014; Fitzherbert et al. 2008; Wilcove & Koh 2010). The implications of this for crocodile nesting are unknown, although crocodiles are frequently seen in plantations (*pers. obs*.). Preliminary data presented in Chapter 4 indicate that crocodiles still do nest in areas of medium to high anthropogenic disturbance, and within close proximity to oil palm plantation. This could be indicative of either insufficient nesting habitat being available or individuals continuing to utilise successful nest sites even after their surrounding environment has been altered anthropogenically. There are numerous other less-well examined effects that could influence both the likelihood of successful nesting, as well as post-natal survival of the estuarine crocodile. Oil palm plantations require a non-natural, irrigation system to ensure sufficient water for crop development; they are, however, unable to withstand long periods of flooding (Abram et al. 2014). These artificial hydrological systems are unlikely to benefit hatchling dispersal and are likely to bring them into closer proximity to potential predators such as monitor lizards (Somaweera et al. 2013). Large quantities of herbicides and pesticides (including Paraquat™ (N,N′-dimethyl-4,4′-bipyridinium dichloride) which has been linked to Parkinson's disease (Dinis-Oliveira et al. 2006; Howard et al. 1981)) are also used in oil palm plantations. These chemicals are highly toxic to all forms of life and have the potential to result in reduced egg and embryo survival.

It is known that utilisation of drone surveys enables the high-resolution identification of crocodile nests; this allows more accurate mapping of their spatial distribution (Chapter 4). This present study aimed to identify all possible estuarine crocodile nests within a specific region of the

Kinabatangan River. In doing this, the hope was to establish baseline data for crocodile nesting in a typical tropical, freshwater ecosystem. Information on the general habitat utilised in nesting sites and how close to anthropogenic activity nesting is occurring was also gathered. Using these data it was hoped to be able to predict whether increased human involvement and association could lead to increased levels of human-crocodile conflict. Nest sites can, as previously discussed, be found in semi-predictable locations owing to their proximity to permanent water sources, as well as their prevalence in certain types of habitat (Harvey & Hill 2003; Magnusson et al. 1978; Webb et al. 1983). Given both the largely closed high-level canopy found throughout tropical rainforest, and the aforementioned predilection for swampland, particular targeting of 'suitable' nesting habitat was possible. Given this directed approach to survey planning, it was hoped that the highest proportion of aerially-visible nests could be located with the minimal survey effort.

The study set out to test a series of hypotheses. Firstly, that estuarine crocodiles actively select non-random nest sites (Hypothesis 1). It was also believed that nest site selection by the female is predictable and can be selectively surveyed through the use of predictive modelling and aerial drone technology (Hypothesis 2). Nest sites are solitary in nature, with individuals actively choosing nest sites spatially independent of other nesting females (Hypothesis 3). Nest site density was higher in exposed, open-canopy areas than under dense forest cover with walked "closed-canopy" surveys justifying the use of aerial reconnaissance (Hypothesis 4), validated the use of drones as a tool for nesting surveys. Finally, nesting can occur in the presence of medium to high levels of human disturbance, this implies that the presence of oil palm plantations is not a barrier to successful nesting (Hypothesis 5).

5.2 Methods

The study was carried out over a 35 km stretch of the Kinabatangan River (see Section 3.2.1 for detailed description). In brief, the second longest river in Borneo, and, at 560 km, the longest in Sabah, the Kinabatangan River has a catchment of around 16,800 km^2, an area encompassing around 23% of the total land area of Sabah (Scott 1989; WWF 2003). Within this catchment area lies the Lower Kinabatangan Wildlife Sanctuary (LKWS), consisting of ten distinct forest 'lots' covering an area of 27,960 ha (Abram et al. 2014). The landscape consists of a highly fragmented forest-oil palm matrix, with forested areas being largely degraded secondary forest. This type of forest results in patchy areas of closed canopy forest, open grassland and areas with very sparse partial tree coverage. Forest conversion is still happening across the floodplain although this is largely confined to privately owned land, outside of the LKWS. The river has retained a large proportion of its original fauna despite widespread conversion to oil palm (Abram et al. 2014). This abundance of wildlife located in close proximity to the river has led to the LKWS becoming a hub for the region's ecotourism. The Kinabatangan region also supports a substantial population of estuarine crocodiles (*C. porosus*); the population has undergone rapid recovery since state-wide protection of the species in 1982 (Sabah Wildlife Department 2010A).

5.2.1 Walked Surveys

Walked surveys were carried out initially, throughout the 35 km study region, to determine whether crocodiles were likely to be utilising areas of closed canopy cover for nesting. The lack of substantial evidence of closed canopy nesting would provide sufficient evidence that aerial nesting surveys had the potential to identify the vast majority of nesting occurrences. These detailed walked surveys, of 10 km in length, were conducted over an area totalling 120 km of riverbank, oxbow lake and swampland habitat. Each survey was conducted by two observers, each of whom walked parallel to

the water source at a distance of 5 m from the water, whilst maintaining 15 m between observers, creating coverage of approximately 20 m (Fig 5.1).

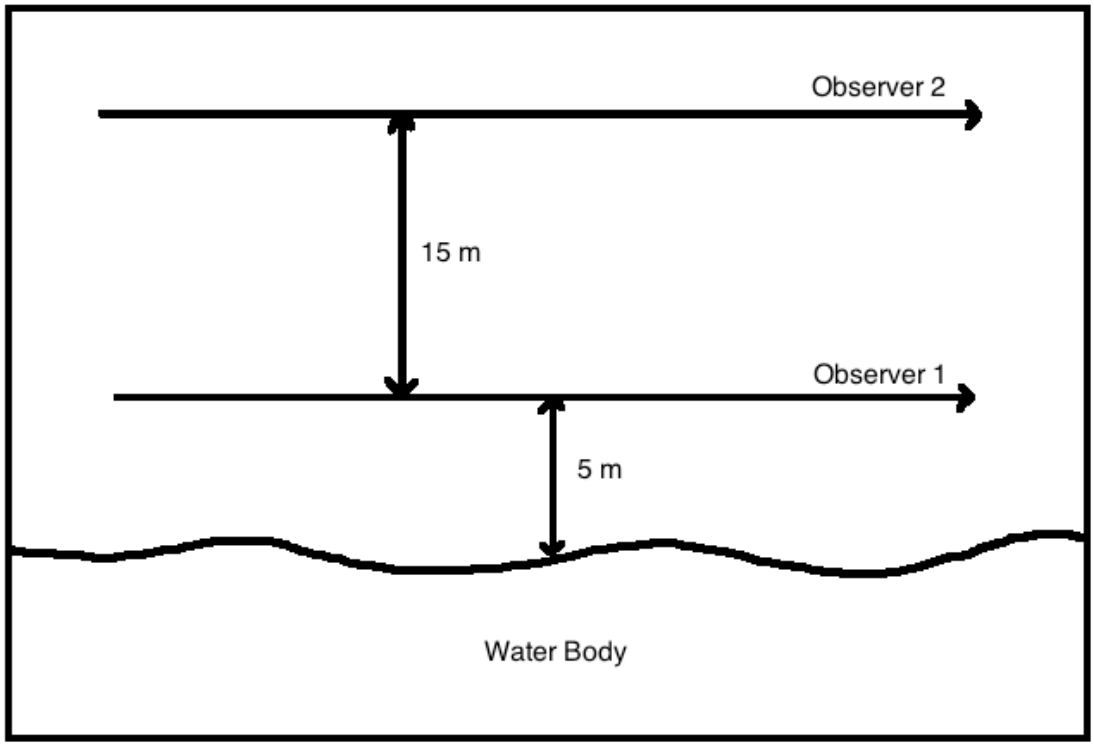


Figure 5.1 – Search methodology employed by two observers carrying out crocodile nesting surveys. Illustrated is the unidirectional approach and how a 20 m search area was covered along the entirety of transects.

Of the 120 km of walked surveys that were conducted, 50.83% of the habitat surveyed was riverbank, 27.08% oxbow lake and 22.08% swampland, across 14 separate transects (Fig. 5.2). Nesting transects (4.5-10 km) (Fig. 5.2) were searched, in detail, for any indication of crocodile nesting or crocodilian presence; for example, footprints or slide marks caused by the dragging of the body through mud, sand or vegetation. Marks found were carefully examined to distinguish them from other animals such as monitor lizards, snakes, bearded pigs or any other ground-dwelling animals found in the region. Transect locations were chosen as being potentially suitable for nesting primarily based on their proximity to permanent water sources (Harvey & Hill 2003; Somaweera & Shine 2012; Webb et al. 1977). Each transect was selected to incorporate the highest percentage of closed canopy possible; this allowed exploration of whether regions with open

access to direct sunlight were more likely to be selected for nesting (Hypothesis 4). If so, such preferential selection also validates the use of aerial detection techniques. The walking of large tracts of the landscape also provided an indication of the different habitats present within the region, and which areas would be most suitable for aerial analysis.

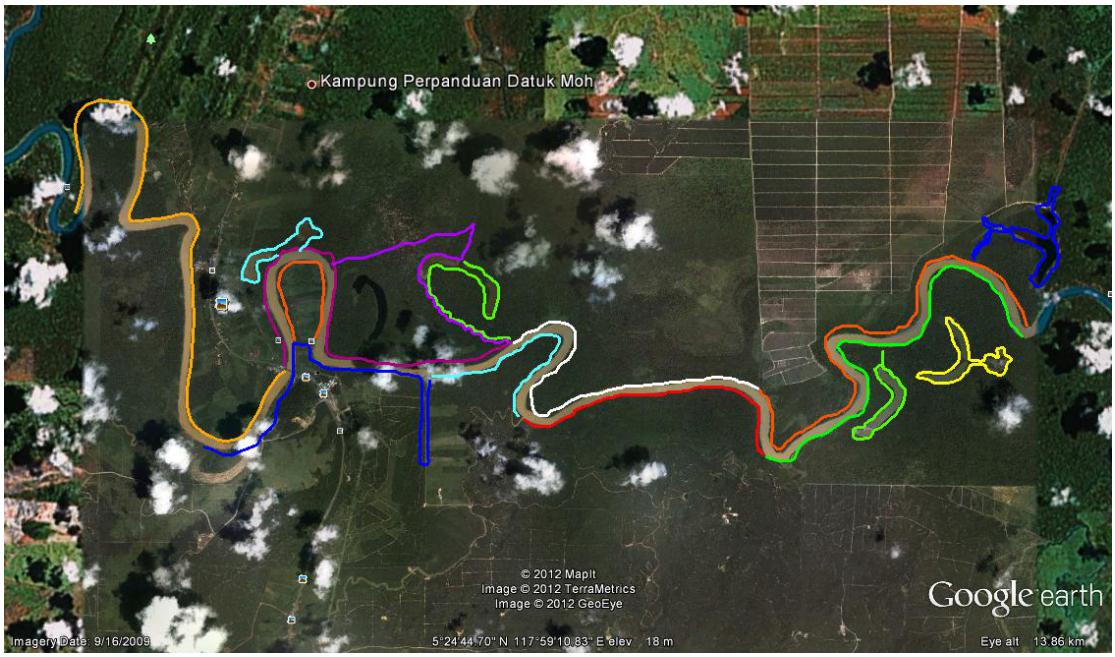


Figure 5.2 – Paths taken during walked crocodile nesting surveys. Transects were searched in a detailed manner by two observers covering a 20 m wide search area (see Figure 5.1). Transect lengths totalled ~120 km, each individual colour represents an individual transect carried out at a different time.

5.2.2 Drone surveys

Aerial surveys were conducted on the basis that nesting under closed canopy was occurring at such low occurrence that there was no meaningful impact on estimated nest densities. Aerial surveys were conducted with the use of two different drone systems (during 2013 and 2014 season, respectively).

In 2013, an exploratory series of surveys were carried out using a Bormatec Maja™ drone (see Chapter 4 for description and detailed reporting of equipment setup). As this was successful, a second, more expansive, series of surveys was conducted in 2014, this time utilising the Skywalker™ drone. This equipment provides a more stable and efficient flight. Both drone

systems were fixed wing aircraft, able to provide a high degree of stability, whilst ensuring desirable range capabilities. Under normal weather conditions (low wind and no rain), the Skywalker™ was able to conduct flights totalling one hour, whilst carrying a payload of up to 1 kg. In real terms, this equates to flight distances of up to 35 km and search grids (see Chapter 4 for explanation of flight planning) of around 550 ha.

Other than during take-off or landing, or during emergencies, drone flights were conducted using an Auto Pilot Module (APM) (see Chapter 4 for more details). Manual flight of the drone required intensive training, first through the use of a flight simulator, and subsequently with a real aircraft in a controlled setting. This preparatory work required around 50 flight hours of training. Simulator training consisted of 10-20 hours to ensure a basic grasp of aerial manoeuvrability. Practical flight training consisted of 40 hours of take-off, landing and free flight practise. Drones were flown in two different modes. Firstly, flights were conducted in manual mode, without computer assistance. Once mastered, flights were also carried out in "fly by wire" mode; this is a setting that allows the on-board autopilot to make corrections and ensure a smoother flight. This assisted flight mode was used in the field at all times above an altitude of 50 m to reduce the likelihood of crashes.

Flights were conducted as close to the planned flight grid as possible, whilst allowing for an open landing and take-off site. These "ground stations" were located in a variety of habitats, including riverbanks, grasslands and oil palm plantations. Ensuring that ground stations were located close to search grids allowed for larger areas to be covered within any particular grid, as battery power was not wasted traveling to and from the study site. Post-hoc analysis of aerial photographs taken involved stitching, as described in Chapter 4.

Selection of drone mapping grids was based on ensuring an array of riverine, swamp and oxbow habitat, as well as covering all of the major

tributaries across the study site. A predictive theoretical model of suitable nesting sites was also produced to aid in the selection of these sites (Fig. 5.3). This model was produced using "fuzzy membership" and "fuzzy overlay" functions in ArcGIS 10; these functions allowed for the designation of certain required prerequisites (such as distance to permanent water sources) for nesting to be feasible, whilst discounting other areas based on presumed undesirable geographic traits (for example proximity to oil palm plantations). These traits were derived from the existing literature (Harvey & Hill 2003).

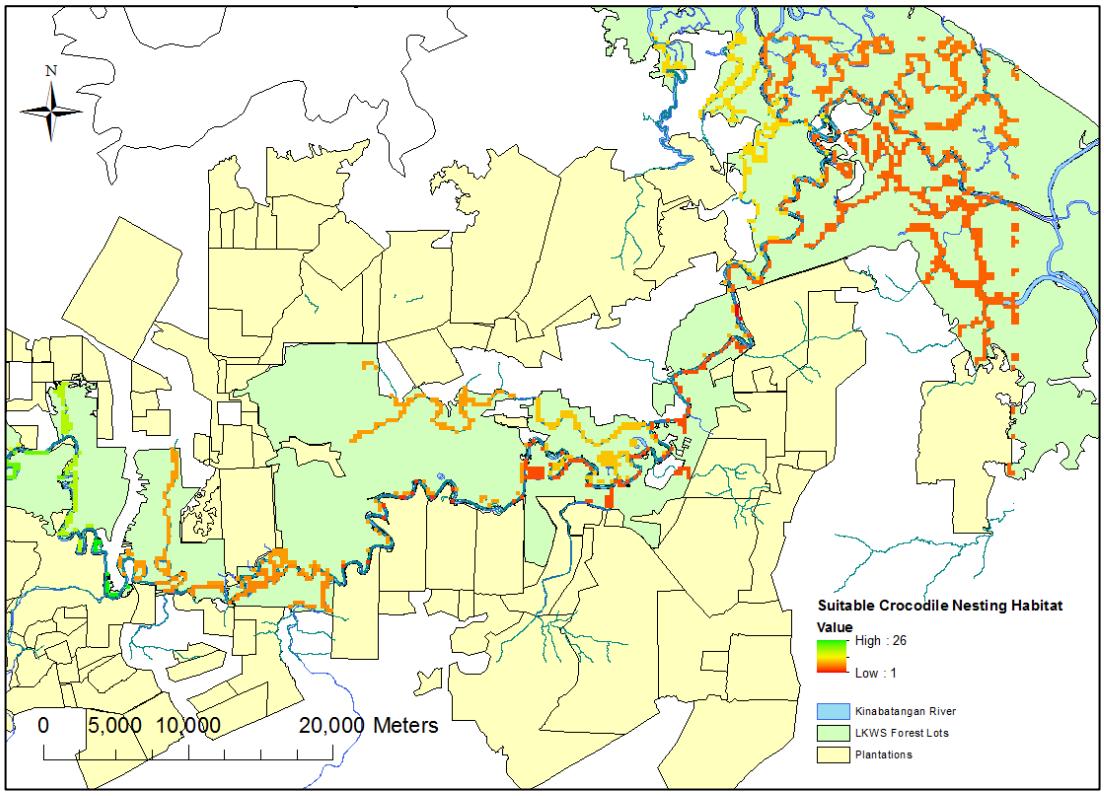


Figure 5.3. Nesting suitability model for the LKWS. Defined using a "fuzzy overlay" model in ArcGIS. Areas of suitability are defined by the presence of a coloured pixel with increasing suitability defined on a green (high) to red (low) scale. Suitable nesting locations are largely confined to major waterways.

Once identified, nests sites were ground-validated, where possible, and general ground habitat assessed. Both validated and non-validated nests were subsequently assessed for a series of geographical traits, such as distance to permanent water, distance to canopy and distance to plantation. These criteria were used to create a generalised linear mixed model (GLMM)

using R (3.1.3). The "lme4" package was used to determine which factors were of the greatest importance in the confirmed nesting locations of estuarine crocodiles. The model was refined through the use of a "dredge" model-comparison function carried out with the use of the package "MuMIn". Conditional and marginal R^2 values were then subsequently used to assess the level of variance explained by both fixed and random model terms. Finally, model predictions were made to evaluate the role proximity to plantations plays in successful nesting efforts.

Table 5.1. "Fixed" and "random" model terms included in the binomial GLMM used to identify the most important factors in the presence or absence of crocodile nests. A logit link function was used for the model.

Dependent Variable	Fixed Model Terms	Random Model Terms
Presence of Nest (1/0)	Ground solidity	Year of detection
	Distance to water	
	Distance to canopy cover	
	Distance to plantation	

5.3 Results

Walked surveys failed to identify any nests, providing evidence that the vast majority of nesting is occurring in areas of open, canopy devoid, areas. During the 2013 field season, a total of 1,550 ha were surveyed using drones and three potential nests identified. Two of these were confirmed as true nesting locations (Chapter 4). A further 5,160 ha was surveyed during 2014; this gave a total area surveyed over two field seasons of 6,710 ha.

Flooding of a large part of the study site during the field season resulted in not being able to verify 10 of the 26 potential nests identified during 2014. Of these ten, nine were excluded from analysis. The additional nest was included as a confirmed nest despite a lack of ground verification based on similarities between its aerial image and those of previously ground verified nests. Those nests that were flooded were not verified either because of safety concerns or lack of accessibility. Of the drone-facilitated nesting

surveys carried out over two field seasons, 2013 and 2014, 29 potential nests were identified. Of these, four were confirmed as actual crocodile nests with the addition of one unverified nest included for the analysis (Fig. 5.4).

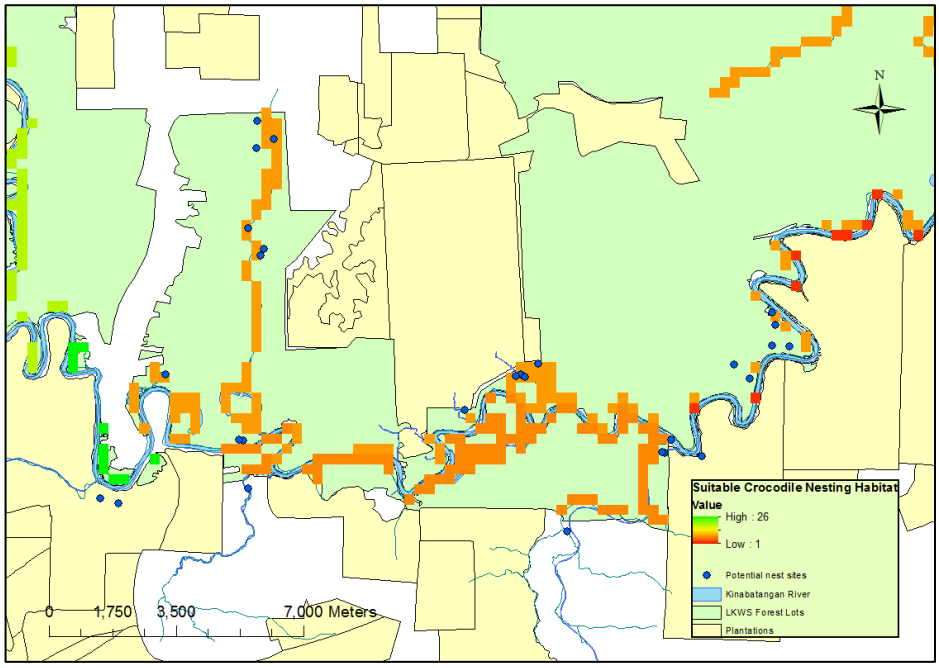


Figure 5.4. Potential nest sites in relation to habitat suitability model; the majority of nests sites fell inside of, or close to, identified suitable areas within the study site. Suitability defined as areas of coloured pixels as in Figure 5.3, with potential nest sites overlaid as blue dots.

Of the five confirmed nests, all were located in close proximity (13.9 m (±12.9 m)) to permanent water sources. The nests were also found within forest habitat (Fig. 5.5), in small open areas and within close proximity (mean (±s.e) 22.2 m (±14.3 m)) of closed canopy cover. Plantations were generally not surveyed but one nest was found close to a plantation border; across all nests identified, the nests were a mean (±s.e) distance of 374 m (±139.7 m) from such boundaries. Four of the confirmed nests were located within the protected habitat lots of LKWS. One nest was located outside these lots, in privately-owned land that could be open to conversion.

Neither of the nest sites located during 2013 were reutilised during 2014. All confirmed nests were spatially independent, although potential nest

aggregations were located and later discounted during 2014 surveys (Fig. 5.5). Three nests were located in “drying” or “old” oxbow lakes; each of these had a permanent aquatic connection to a main water body, such as a large tributary. The other two nests’ locations were directly adjacent to a major water body.

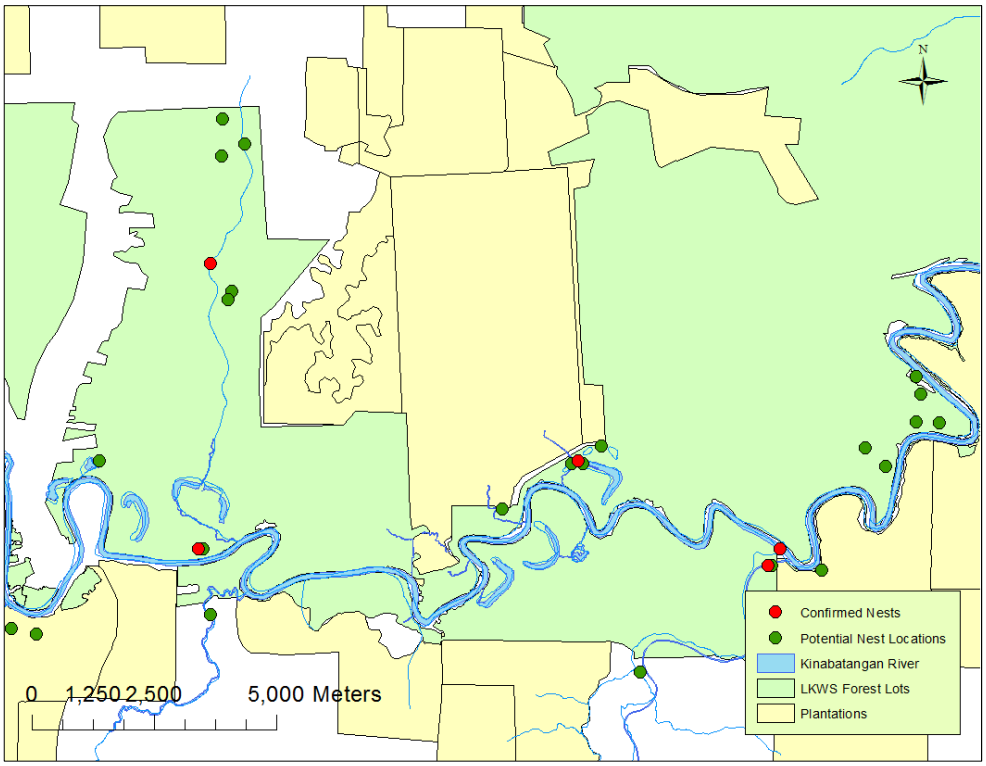


Figure 5.5. Locations of confirmed nest sites showing close proximity to water, as well as, on three occasions, close proximity to oil palm plantations. Includes identified nest sites from Figure 5.4.

Nest sites could not be attributed to specific females and no instances were recorded of females guarding their nests. There was, however, evidence of females spending time at the nest site and of excavation of eggs during hatching, as well as wallows around one of the nest sites. One nest located during 2013 surveys was visited the day after hatching and visual confirmation of 19 hatchlings was recorded (Fig. 5.6). Egg membranes and shells were collected, and evidence of at least 24 successful hatchings were found. There was no evidence of pre- or post-natal mortality.

Figure 5.6 a) Day old hatchling from nest located aerially through the use of a drone. b) Evidence of one of 24 egg membranes recovered from the nest site.

Two of the confirmed nests fell within the territory of a female that was satellite-tagged (Female 1 (F1), see Chapter 3). Although it is unlikely that both nests belonged to F1, there did appear to be some spatial overlap between one of the nests (Fig. 5.7 gives spatial data for F1); the aggregation of points is far less than one would expect of a guarding female. The fact that two nests exist within the range of one female suggests that females are not spatially-exclusive or territorial.

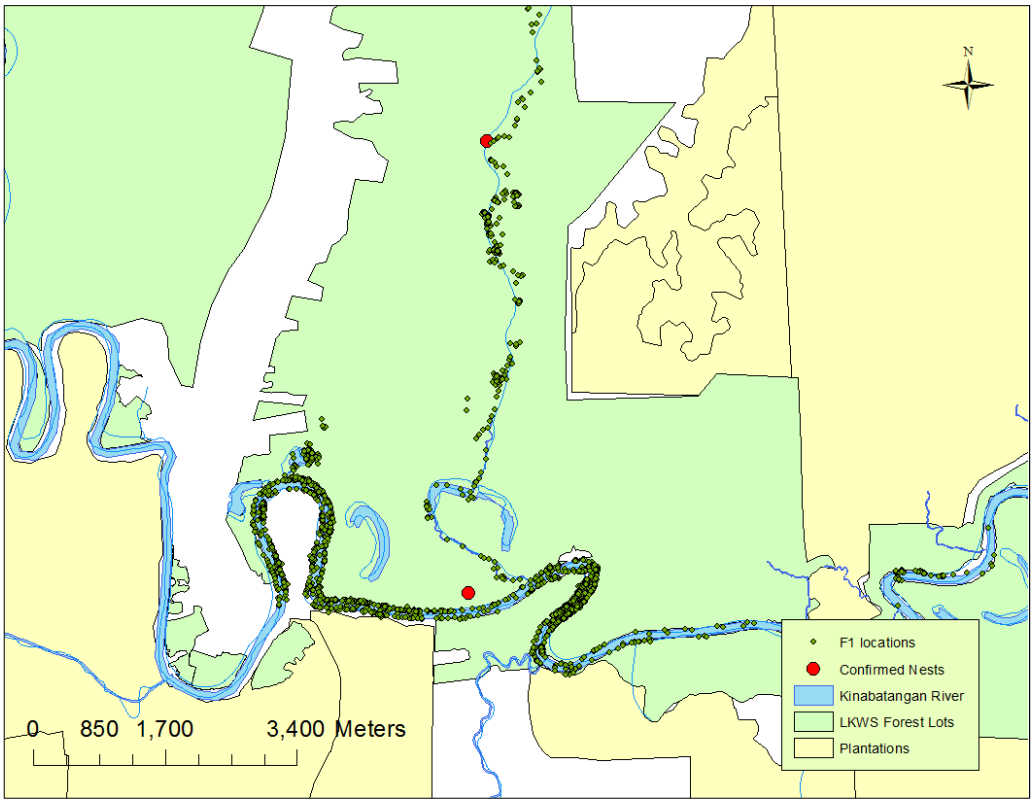


Figure 5.7. Presence of two aerially confirmed nests found within the territory of a single satellite tagged female (F1). Green points refer to spatial locations of F1 and red points the locations of confirmed nest sites.

The most parsimonious GLMM model structure to explain the presence/absence of crocodile nests (lowest AIC – Akaike Information Criterion identified using the "dredge" function) included the variables "distance to water" and "ground water presence". This model also yielded the greatest model weight (W = 0.195), indicating that this model structure best explains the included model variables. Distance to water was found to be significantly negatively correlated with the likelihood of finding a nesting site ($F_{1,29} = 5.59$, $p = 0.018$). Additionally, the presence of ground water was also found to be close to significance ($F_{3,29} = 7.36$, $p = 0.061$), with the presence of less than 1 m of standing ground water resulting in a higher likelihood of nesting. The addition of further data would possibly result in this variable becoming significant. Marginal (R^2m) and conditional (R^2c) r-squared values showed that the vast majority of the variance being described by the model was derived from the fixed terms (distance to water and standing ground water), with negligible variation explained by the random term, year ($R^2m = 0.571$, $R^2c = 0.571$).

Predictions based on this model resulted in an indication that whilst locations of nests in standing water could not be easily predicted, nests on solid ground were very likely to be less than 100 m away from permanent water sources (Fig. 5.8). In reality, these predictions are in line with those used during the original "fuzzy overlay" modelling (Fig. 5.4). An increase in sample size could lead to a refinement of solid-ground predictions and lead to more stringent standing water predictive sampling.

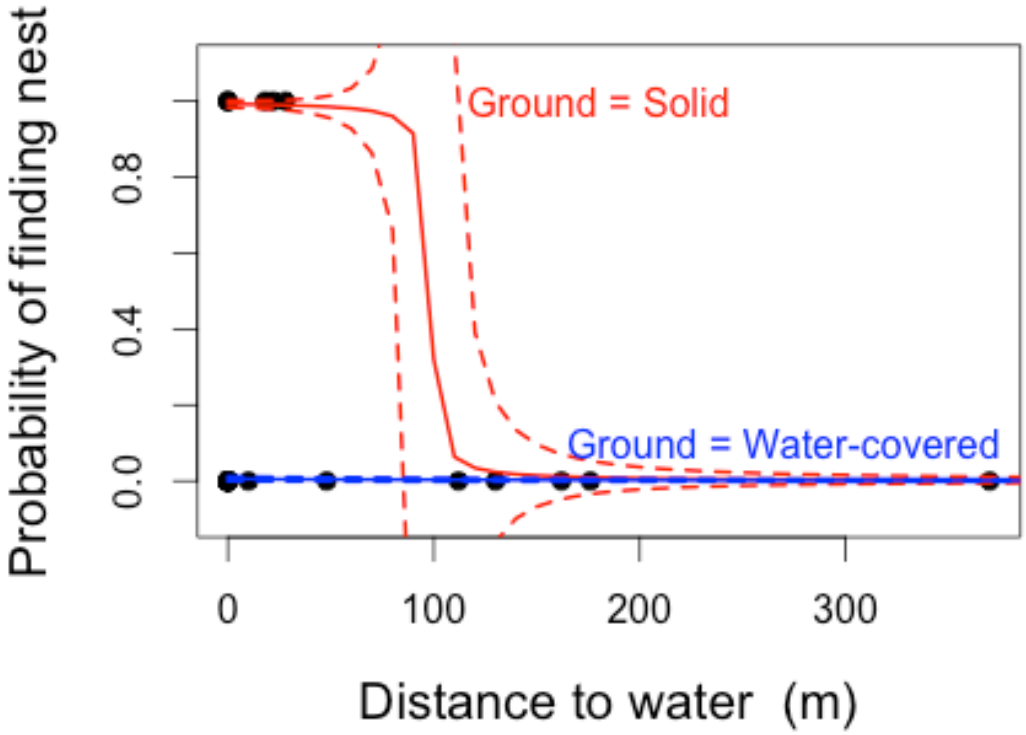


Figure 5.8. Plotting predictions from binomial GLM. Model provides a Binomial predictive distribution, indicating that nesting is less likely further away from permanent water sources. The model does not provide predictive information for instances of water-covered areas. Data included both confirmed nest sites as well as those that were "potential" and later discounted nest sites. Despite trajectory of confidence intervals, prediction could not be less than zero.

Figure 5.9. The difference in resolution between 2013 surveys (a) and 2014 surveys (b and c). Images show the same ox-bow lake; both images were zoomed to the same extent. Both (a) and (c) show the difference in image clarity between confirmed nests. Flights were carried out using different cameras of the same make and model.

There was a marked difference in image quality between the 2013 (higher quality) and 2014 (lower quality) surveys. This was despite the use of a camera of the same make and model, albeit a different camera. This could also have been a result of different lighting conditions (see Chapter 4), or the speed of travel of the drone.

5.4 Discussion

In Chapter 4, the feasibility of using drones to detect crocodile nests was demonstrated. In the present chapter, where use of the technology to carry out a survey of a section of the Kinabatangan River is reported, verification was of only five nests of a total 29 potential locations. The approach, albeit in need of improvement, is clearly a major improvement on the traditional techniques of, for example, helicopter surveys entailing flying over large tracts of unsuitable habitat and still requiring post-event ground validation. That five nests were identified from the sampling of a relatively small (6,710 ha) selected area of the study site suggests that females are actively selecting areas identified from the habitat suitability model (Hypothesis 1). The use of a habitat suitability model, as well as selection of areas with open or semi-open canopy coverage, allowed for both highly-selective and highly predictive mission planning (Hypothesis 3). The walked surveys provided important justification of the use of drones in identifying potential nesting sites and crocodilian habitat (Hypothesis 2), and although the distance walked was relatively short (120 km), the transects' placement to encompass tributaries, ox-bow lakes and other areas of permanent water sources (essential to successful nesting), provided a clear indicator that dense forest canopy does not provide an important nesting habitat. This is due to the fact they were largely conducted beneath closed canopy (Hypothesis 4). The identification of nests in close proximity to oil palm plantations, as well as the confirmation of two nests within plantations outside of the study area, demonstrates that females are capable of nesting in medium to high levels of human disturbance (Hypothesis 5).

While the identification of five nests over the two field seasons provides a degree of validation of the methodology, the limited 35 km river stretch study site probably represented too small of an area to provide a clear picture of the nesting habits of the crocodiles found throughout the LKWS. Nesting appears to be occurring at low densities, and whilst this nesting is, at least to some extent, predictable, the presence of degraded, patchy

secondary forest represented a challenge to successful and encompassing nesting surveys. Secondary forest results in far larger expanses of open areas than would likely be present were the region to still retain its original primary forest landscape. As a result, refining search grids is more challenging in secondary forest ecosystems than in pristine primary forest habitats. Despite this reduction in canopy coverage, it is unlikely that all nests will be built in open areas and crocodilians can use alternate heat sources such as termite mounds to keep their nests at the optimal temperatures (Magnusson et al. 1985). The effect of this on the number of nests detected should, however, be negligible.

Flooding, as also determined by Webb et al. (1977), is the primary threat to *C. porosus* nests in the Kinabatangan. Ten potential nests were completely submerged during the period of ground-verification after the river rose in excess of 1 m over one night. This flooding did, however, lead to the identification of one "floating" nest that had been built to reduce the possibility of mortality due to flooding. Floating nests are widely reported across many crocodilian species, including *C. porosus* (Campos 1993; Somaweera et al. 2013); the nests generally consist of floating mats of vegetation (Campos 1993). In the current observation, the floating nest did not perform as expected. All eggs were recorded as being under water (*pers. obs*.).

Application of a GLMM was intended to inform what a model habitat would be for crocodile nesting in the LKWS. The model predictions provided less stringent buffers around major water sources than used in the original habitat suitability model and, as a result, the original cut off of 150 m (derived from the literature) used during the original "fuzzy overlay" model remained the best predictor of nesting habitat presence or absence. That only five nests were positively confirmed provided a too limited framework to generate statistically rigorous data for generic habitat features. Similarities between nest site choices and their spatial separation determined using the GLMM and from observations, suggests that the

crocodiles of the LKWS are choosing sites preferentially and making active selections for nest locations (Hypotheses 1 and 3). That the nests of the 2013 survey were not reused, despite being successful (Fig. 5.6), suggests either that females are not nesting annually or are not nest site-fidelic. There appeared to be a general preference for smaller open areas, rather than large expanses of open grassland or swamp.

Outside the study area, one nest was located within a long-standing plantation, in an area that had been converted from forest at least 25 years previously. This area was visited and nest presence confirmed (Fig. 5.10). There was evidence that the site had been used for nesting on more than one occasion, and conversations with local plantation workers suggested that nests were constructed on this site on a biennial basis. Older plantation workers claimed that this nest site had been used since the construction of the plantation. Such behaviour suggests that females may revisit successful nest sites regardless of a change in localised habitat and this would explain why areas along the Kinabatangan River, devoid of forest fragments, still harbour significant numbers of hatchlings. Individual crocodiles appear able to nest within areas of high human disturbance across multiple nesting seasons (Hypothesis 5). The lake within which the nest was located was also observed to contain hatchlings of different age classes; this suggests that nesting events were successful on multiple occasions. The resilience of individuals to nest in suboptimal areas, as long as these locations meet basic nesting requirements, could be a key factor in ensuring species perseverance. Nesting in plantations does, however, raise the likelihood of human-crocodile conflict; this was evidenced by the nest under discussion (Figure 5.10), which, according to local inhabitants, has been a site of human-crocodile conflict within the last 10 years, although, at least to date, no injuries have been sustained.

The 'identification' of plantation nest sites some distance away from the Kinabatangan River provides an important challenge to the use of drone technology. Plantations are not only difficult to survey aerially due to their

semi-open nature, but also, human presence and activity can give rise to "non-natural" formations, usually created by plantation workers, that are identified, incorrectly, as potential nests. While drone technology has undoubted applicability in highlighting key areas of nesting habitat, density estimates garnered in this way should be tempered with extreme caution.

Figure 5.10 a) Nest located in shallow lake showing central mound of vegetation surrounded by water of 1 m depth. b) Claw and slide marks indicating female willingness to cross plantation roads to get to the nest site. Arrow indicates claw sliding as the hill is scaled by the female.

The increase in human-crocodile conflict incidences in the surrounding region (Sideleau & Britton 2012) has the potential to undermine conservation efforts for a species that has undergone a dramatic recovery in the 33 year period since given protection status in Sabah. Growth in both human and crocodile populations require the animals to be of intrinsic value to local people. One way of achieving this is through eco-tourism. Crocodile-based eco-tourism has become widely successful in Northern Australia and could provide a model for how Sabah could utilise its crocodiles, in conjunction with the plethora of other species found in the state, to create an eco-tourism hub.

In carrying out this study, numerous UAV (Unmanned Aerial Vehicle)-related challenges were faced; as a burgeoning technology there are still some aspects of its use that need further development. While repeatability of transect observations is one of the major benefits of the technology over traditional techniques such as helicopter or airboat surveys, the huge

disparity in the resolution of the images produced in the two survey years (Fig. 5.9), although the same make and model camera (Canon S100) was used, is of concern. This variation could have resulted in the omission of potential nest sites during the 2014 surveys. Conversely, low-resolution images meant that a much larger number of "potential" nest sites needed to be ground-verified, as they could not be excluded due to poor image quality. A similar image resolution of 5-6 cm per pixel to that which was achieved during the 2013 surveys would have allowed for the exclusion of a number of the 2014 'potential' nests. Drone flight capabilities are constantly being developed and improved, and with aerodynamic technology advances and, more importantly, improved battery efficiencies, flights of up to 70 km are already possible.

The crocodile population of the LKWS has endured fluctuations in both extent and stability; the current size of the population has, however, raised human-conflict concerns, with six known fatalities having occurred within the study area since 2010. The mapping of nesting habitats has a role in the mediation of conflict zones, especially if a further reduction in habitat results in a closer nesting proximity to human settlement. The identification of nests on an annual basis can also aid in the mapping of population trends. This, coupled with spotlighting surveys, could give a better indication of how the population is adapting to anthropogenic expansion. Nesting surveys of this nature could also provide an idea as to the carrying capacity of both the study site and the LKWS as a whole, and how crocodile number could alter as forest conversion continues. An increase in sample size provided by annual nesting surveys would allow not only for a more in-depth modelling of nesting areas but also a more stringent predictive modelling. In this way, areas deemed most important to successful nesting could be protected, and this, in turn, providing mediation of human-conflict issues.

Whilst in terms of cost, drone technology is far cheaper than many traditional survey methodologies, the main barrier to its use by small-independent research projects is the cost of image stitching. Whilst the

financial implications of each individual flight is relatively low, the stitching costs are beyond the budgets of some small organisations (a cost of around GBP1 per hectare (total area surveyed 6,710 ha)). These data can be collected without the use of image stitching but detailed analysis of each specific image would require a far longer time-period. Not only would the identification of nests prove more difficult but placing the location within the broader context of the landscape, and assessing the hydrological relations would be far more challenging and would require a highly specified knowledge of the study region.

In summary, the nests identified were spatially exclusive, showing that *C. porosus* individuals in the LKWS are not aggregate nesters. There was also no evidence of annual reuse of nesting sites. There was, however, an element of predictability to nesting site location, in relation to distance from water bodies, that could be modelled, thus allowing search area refinement. Nests were located at least several hundred meters from each other and from any previously-used nesting sites. Nesting sites were found at medium-disturbance level sites, although the presence of nest sites inside plantations from outside the study site indicated that even daily human disturbance is not necessarily a barrier to nesting. This does suggest that stable populations could endure, even in areas of extreme land-use conversion. The presence of floating nests suggests an adaptation to local climactic conditions with areas less suitable for nesting being utilised, a crucial adaptation in areas of lessening habitat availability.

Chapter 6 - Population Genetics of the Estuarine Crocodile, *Crocodylus porosus*, in Sabah, Malaysia.

6.1 Introduction

Understanding the role that genetics play in the management of a species has become a crucial component of conservation strategy. As well as playing a vital role in the identification of important population structures, or Evolutionary Significant Units (ESU), in many organisms (Ciofi & Bruford 1999; Ciofi et al. 2002; Moritz 1994; Segelbacher et al. 2014; Shamili et al. 2012), genetics and, in particular, population genetics dealing with the variation in allelic frequency changes (Manel et al. 2003; Sugg et al. 1996) has become an essential facet of conservation planning. The understanding of the genetic implications of population recovery has led to both the successful management and on-going rehabilitation of many crocodilian populations following the huge declines recorded throughout the 20th Century (Fitzsimmons et al. 2000; Thorbjarnarson et al. 1998). Globally, with six species remaining listed as critically endangered, understanding the genetic health of these species is of paramount concern to their continued survival and management.

As previously mentioned (Chapter 2), the estuarine crocodile (*Crocodylus porosus*) is the largest extant crocodile in the world, reaching, length-wise, in excess of 6 m (Britton et al. 2012). It also has the broadest habitat range (Australia to eastern India) of any extant crocodilian (Russello et al. 2007). Estuarine crocodiles can be found throughout the south-east Asian region, from India in the north, to Australia in the south (Anuar et al. 1996; Hanson et al. 2015; Lewis et al. 2013). There are, however, areas throughout this range where populations have been either severely depleted or completely extirpated (Brazaitis et al. 2009). The estuarine crocodile was, some 40 years ago, considered to be endangered and at risk of extinction (Bustard 1970). Legislative protection and active conservation have led to the downgrading of *C. porosus* on the IUCN Red List to “least concern”. Genetic

analysis has the ability to quantify the effects of population fluctuations which occurred across the Crocodylia in the 20th Century. The understanding of the implication of genetics in the survival of a species has become increasingly apparent over the last few decades, and the field of conservation genetics has resulted in the successful management and rehabilitation of many species (Frankham 1995; Rubinoff 2006). Fitzsimmons et al. (2000) discussed the possibility that estuarine species, such as *C. porosus*, might have increased gene flow (transfer of alleles between adjacent populations) when compared with freshwater species; this is thought to be due to the former's ability to transverse oceanic waters. High levels of gene flow could be partially responsible for the recovery of *C. porosus* that was experienced following the cessation of hunting activities (Fukuda et al. 2011).

Crocodylus porosus is one of several species of crocodilians with multiple microsatellite loci having been identified. Others include the American alligator (*A. mississippiensis*), the broad snouted caiman (*Caiman latirostris*), the Morelet's crocodile (*C. morletti*) and the Australian freshwater crocodile (*C. johnstoni*)(Miles et al. 2008). Loci have been identified that measure multiple traits, including population diversity and mating behaviour (Isberg et al. 2004; Miles et al. 2008). The latter authors, for example, identified 253 new microsatellites for the estuarine crocodile, and these, because of the high levels of genetic diversity exhibited in many microsatellite loci (Bruford et al. 1996; Wright & Bentzen 1994), can be utilised to identify not only the genetic diversity present in a given population, but also to model the history of the population and to estimate effective population size.

Most population genetics studies of the estuarine crocodile, both on captive and wild populations (for example; Isberg et al. 2004; Jaratlerdsiri et al. 2012; Miles et al. 2010; Miles et al. 2008; Miles et al. 2009), have been carried out in Australia. There have been numerous studies examining the population genetics of various species of crocodilian, such as Morlet's crocodile (*C. moreletii*) (Dever et al. 2002; González-Trujillo et al. 2012),

American alligator (*A. mississippiensis*) (Glenn et al. 2002) and American crocodile (*C. acutus*) (Cotroneo, 2010), broad-snouted caiman (*Caiman latirostris*) (Saidman et al. 2012) and the Nile crocodile (*C. niloticus*) (Hekkala et al. 2009; Schmitz et al. 2003). A number of population genetic studies that have focussed on *C. porosus* have been restricted to farmed, captive bred populations (Isberg et al. 2004). One major study carried out on the population genetics of wild populations of *C. porosus* occurred in Palau, a small island chain forming part of the larger Caroline Islands in Micronesia (Russello et al. 2007). The authors used samples taken from Kalimantan (Indonesian Borneo) as well as other regions throughout *C. porosus*' range. A single haplotype, also present in Borneo, was found in Palau as well as other locations. This suggests that despite the large species-level range of the estuarine crocodile, there is little in the way of geograpically mediated genetic variation between populations.

Analysis of parentage of wild populations allows for the assessment, and to increase the understanding, of many behavioural and ecological systems. A number of parentage studies have been carried out on *C. porosus*, particularly on captive populations (Isberg et al. 2004). These studies have been routinely used to determine which one, of a series of candidate fathers, has sired a particular offspring. Evidence of mutiple parternity has been demonstrated in a number of crocodilians (Amavet et al 2008; McVay et al. 2008; River et al. 2001), including *C. porosus* (Lewis et al. 2013). To date, however, there remains a dearth of studies using parentage to examine the social ecology and juvenile dispersal techniques within Crocodylia.

Over the past 50 years, C. *porosus* numbers in Sabah have fluctuated, falling to a level that was of sufficient concern to be given a range-wide IUCN Red List listing of "endangered" in 1982 (Crocodile Specialist Group 1996). In the same year, Sabah gave its *C. porosus* populations state-wide government protection and, to the present, they remain on Sabah's Appendix II of threatened species (Sabah Wildlife Department 2010B). To date, there has been no examination of neither *C. porosus* genetics in Sabah nor the impact

of the recent population bottleneck. The present study is a first attempt at answering some of these questions.

The level and rate of habitat alteration and logging occurring throughout Borneo is far more severe than in other tropical regions (Gaveau et al. 2014). The role that habitat fragmentation plays in the behaviour and ecology of an aquatic, mobile predator such as the estuarine crocodile has been little examined and the genetic implications of restricted movements remain un-quantified. The detection of apparent human-mediated barriers to movement, as found in the Kinabatangan (see Chapter 3), raises important considerations for future management strategies. A detailed understanding of the genetic health of the extant population can provide an essential baseline for future studies.

The work reported in this chapter seeks to provide this baseline knowledge of the genetic health of the Kinabatangan crocodiles following the population bottleneck that occurred during the 20th Century. In doing this work it is hoped that the population structure and overall diversity of what is believed to be the largest population of estuarine crocodiles in Sabah, can be explored to aid in the future management of crocodiles in the region. By assessing the genetic differentiation of a major population of estuarine crocodiles in Borneo it is also an intention to establish whether the region is one of special conservation importance for the species. Using relatedness and parentage analyses it is also hoped to increase understanding of mating relationships and juvenile dispersal patterns of this crocodile species.

6.2 Methodology

6.2.1 *Field Sampling*

Tissue samples were collected from 122 unique crocodile individuals between March 2012 and December 2014 across an approximately 200 km length of the Lower Kinabatangan Wildlife Sanctuary (LKWS) (Fig. 6.1). Samples were mostly collected from the Kinabatangan River (*n*=111) and

from a crocodile farm located between Kinabatangan and Labuk Rivers (*n*=11) (~N5.546456, E118.013712). Individuals sampled in the Kinabatangan River ranged in size from 30.2 cm to 518 cm; this included 30 adults and 64 first-year hatchlings, with the remainder being sub-adults. Although no reliable information on capture locations was available, the majority of the farmed individuals were presumed to have originated in the Kinabatangan River. They ranged in size from 2.54 to 3.81 m. Adult individuals were captured using steel mesh traps (see Section 3.2.2), whereas juveniles (shorter than 1.5 m in length) were hand-captured from a boat. These smaller crocodiles are easier to handle and carry a much-reduced risk during capture (for both animal and handler).

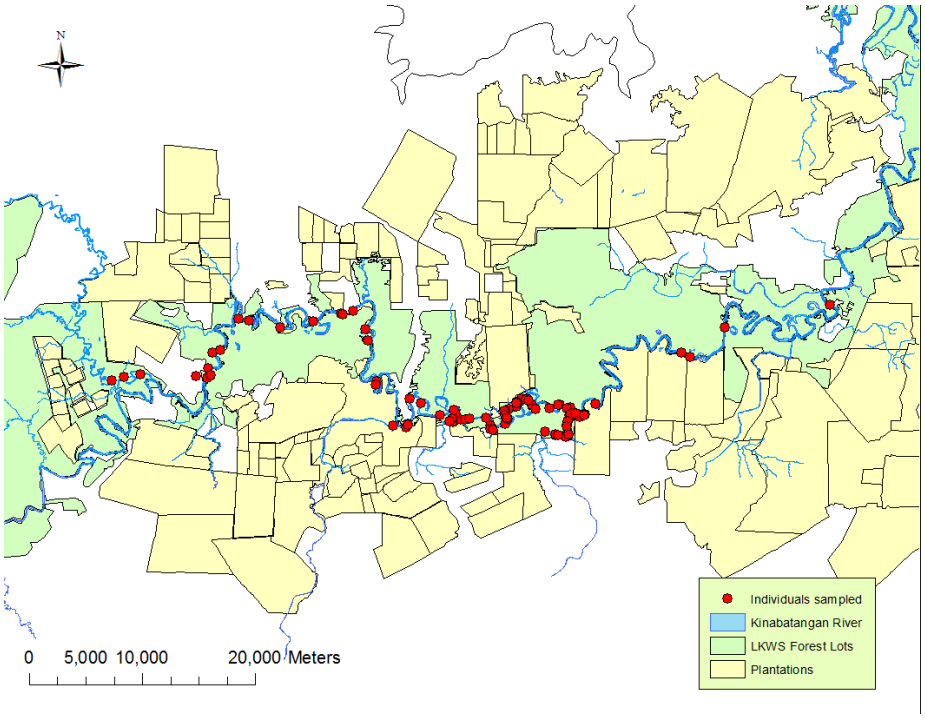


Figure 6.1. Capture locations of all 111 wild-caught individuals sampled (each red point indicates a single unique individual sampled). A further 11 individuals, from a crocodile farm, were sampled upon seizure. Showing the ~200 km extent of river habitat surveyed during the course of the study.

Table 6.1. Summary of selected morphometric measurements across all individuals (n=122) sampled. Morphometric measurements included total length (length), snout to vent (SV) and head length (Head).

	Mean (cm)	SE (cm) (+/-)
Length	115.63	120.17
SV	46.43	52.13
Head	19.85	21.68
Head to TL ratio	4.87	0.38

Sub-adult individual captures were carried out at night; eye shine was used to identify a crocodile's presence. Hand capturing involved grasping the animal at the base of the skull with a firm but not constricting grip. Handling of hatchlings had to be carried out with care so as not to damage the fragile limbs. Upon capture, individuals had their mouths secured and morphometric measurements taken (Table 6.1). Tissue samples were taken by removing the tip of a scute (osteodermal ridge) from the mid-section of the right hand side of the tail (Fig 6.2). Adult samples were collected in a similar manner, although just a scute tip was removed (see Chapter 3 for a detailed explanation of adult capture and restraint). Tissue samples were stored in 95% ethanol and later frozen at -86°C.

Figure 6.2. Scute removed from the right hand side of the tail for genetic analysis (red arrow). The scute does not grow back and allows for identification of previously caught individuals.

6.2.2 *Laboratory Methodology*

DNA extraction was carried out in using a Qiagen DNeasy blood and tissue kit (Qiagen, Venlo, Netherlands). A section of the mitochondrial genome's control region (Domain I and II) (~760bp) was amplified through polymerase chain reaction (PCR) using primers L15463 (5' CGCTGGCCT-GTAAGACAGA 3') and H16260 (5' CACTAAAATTACAGAAAAGCCGAC 3') (Fitzsimmons et al. 2002). A total of 30 strategically selected samples were sequenced, selected to cover a range of size and sex demographics from the total sampled populations. The protocol for PCR reactions was as follows: reactions of 15 µl were performed using 2 ng DNA, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.2 mM of each primer (L15463 and H16260), 1X GoTaq® buffer and 0.02 U/µl GoTaq® DNA polymerase (Promega). The PCR thermal cycle conditions were: initial denaturation at 95°C for 2 minutes, followed by 32 cycles of 94°C for 25 seconds, 48°C for 45 seconds and 72°C for 45 seconds. A final extension temperature of 72°C for 5 minutes concluded the amplification. Sequencing was performed by First BASE Laboratories Sdn Bhd (Malaysia).

In addition to the 122 samples collected during this study, genotypes of *C. porosus* individuals from across the species range, previously uploaded onto Genbank, were also used for comparative purposes. These included individuals from Queensland (AF542533), the Northern Territory (JQ237683, AF542543, JQ237684 and JQ237685), Australia, and Southeast Asia (AF542535, AF542536, AF542537, AF542538 and AF460213),

The total sample set of 122 individuals were genotyped using 18 published microsatellite primers from Miles et al. (2009) and Fitzsimmons et al. (2000) (Table 6.2). Highly polymorphic loci were selected with the aim of enabling the identification of individuals with high probability (mean (±s.e) k = 10.4 ±5.2). The PCR protocol yielded reaction volumes of 10 µl and included 5 µl of Master Mix (Multiplex PCR Kit, QIAGEN) 0.2 µl of each primer, as well as 2 ng of DNA. Gradient PCR was carried out on each primer using two samples to establish the most efficient annealing temperature,

ranging from 54°C to 64°C in 2°C increments using the following conditions: initial denaturation at 95°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, various annealing temperatures (54°C-64°C) for 1 minute, and 72°C for 90 seconds. A final extension was carried out at 72°C for a period of 10 minutes. Once optimum annealing temperatures were established, microsatellite primers were multiplexed using Qiagen multiplex PCR kit (QIAGEN, Venlo, Netherlands). Microsatellites were labelled with one of three fluorescent labels and the 18 primers separated into four different multiplexes and one uniplex. Multiplexes were assessed with Auto-Dimer (Vallone & Butler 2004) to ensure no primer dimer formation during the PCR. Fragment analysis was carried out by First BASE Laboratories Sdn Bhd (Malaysia).

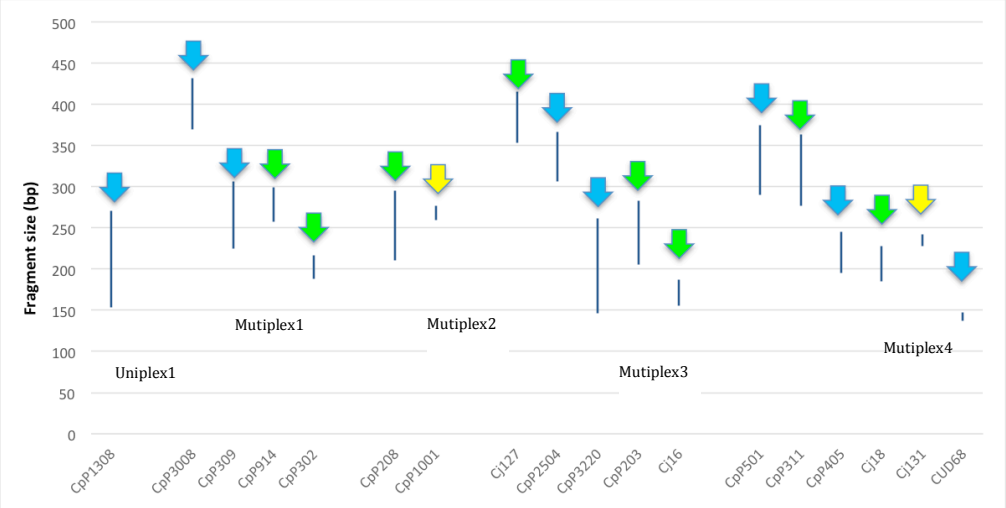


Figure 6.3. Allele size ranges and multiplex assignment. Fluorescent dyes assigned to each primer indicated by coloured arrow: FAM (Blue), HEX (Green) and TAM (Yellow).

Table 6.2. Microsatellite loci utilised across the 122 *Crocodylus porosus* individuals captured and sampled. Included are: primer sequences, repeat motifs, number of alleles (k), allelic range and gradient PCR determined annealing temperatures.[1]-Miles et al. 2009; [2]-Fitsimmons et al. 2002.

Locus	Primer Sequence	Repeat Motif	k	Allele Range (bp) (published)	Annealing Temp. (°C)
CpP1308[1]	F CAGTCGGGCGTCATCAACCTGAAAATGGATACTG	$(AAAC)^{25}$	8	153–271	54
	R GTTTACGCTTGTTAACTTCACT				
CpP3008[1]	F CAGTCGGGCGTCATCAACAACTGGCACATCTCA	$(ACAG)^{17}$	9	370–432	62
	R GTTTCCCGTAGCCTCCTACTG				
CpP309[1]	F GTTTAATACCTGGCATGTGTTCTTC	$(AAAC)^{28}$	23	225–306	62
	R CAGTCGGGCGTCATCACATCAGGTTGGCATTTCA				
CpP914[1]	F CAGTCGGGCGTCATCAACATGGCAACTTCAGAG	$(AGAT)^{9}$	8	257–299	62
	R GTTTCGAATAAATGCAGCATAA				
CpP302[1]	F GTTTGGAACCCAAGAACTTACAAC	$(AC)^{17}$	9	188–217	62
	R CAGTCGGGCGTCATCATTGGGTTTAGTCAGCACATA				
CpP208[1]	F CAGTCGGGCGTCATCACACATGGCTTTTGTTCTGAG	$(AGAT)^{11}$	11	211–295	62
	R GTTTCCTGCAAAATGTTCTCCTA				
CpP1001[1]	F CAGTCGGGCGTCATCAGCAGAAACGAAAGATGTAGT	$(AGAT)^{7}$	3	260–277	62
	R GTTTGGTTCCGTTGGTTTATT				
CpP2504[1]	F CAGTCGGGCGTCATCACTCATATTTCCCAACTATCAC	$(AGAT)^{9}$	8	306–367	64
	R GTTTCATTCCCACAATACACATAA				
CpP3220[1]	F CAGTCGGGCGTCATCAGGAATTGGAGGAATCAGT	$(AAAC)^{25}$	18	146–262	64
	R GTTTGGCCAATGCTCTTTTA				
CpP203[1]	F CAGTCGGGCGTCATCAGTCCATTGCCAGTTGTAA	$(ACAG)^{21}$	10	205–283	64
	R GTTTCTGAGGGTGAACTTTAGAA				
CpP501[1]	F CAGTCGGGCGTCATCACCTGATAGACTGCCTACAA	$(ACAG)^{15}$	15	290–375	64
	R GTTTGTTAGTTCCCACTGAAGAAG				
CpP311[1]	F GTTTGCGCACACACTATATCA	$(AAAC)^{28}$	17	277–363	64
	R CAGTCGGGCGTCATCATAACATGGCAACACATTT				
CpP405[1]	F CAGTCGGGCGTCATCAAGTTTCAGCCAGCTCTAGAA	$(AAAG)^{15}$	8	195–245	64
	R GTTTCCAGAAGCCACCTAAAGTTA				
Cj18[2]	F ATCCAAATCCCATGAACCTGAGAG	$(CA)^{21}$	5	190-192	64
	R CCGAGTGCTTACAAGAGGCTGG				
Cj131[2]	F GTTTGTCTTCTTCCTCCTGTCCCTC	$(CA)^{14}$	9	210-218	64
	R AAATGCTGACTCCTACGGATGG				
CUD68[2]	F GCTTCAGCAGGGGCTACC	$(CA)^{15}$	7	137-147	64
	R TGGGGAAACTGCACTTTAGG				
Cj127[2]	F CCCATAGTTTCCTGTTACCTG	$(CT)^{7}TT(CT)^{12}(CA)^{16}$	10	336-353	64
	R GTTTCCCTCTCTGACTTCAGTGTTG				
Cj16[2]	F CATGCAGATTGTTATTCCTGATG	$(CA)^{20}$	7	132-152	64

6.2.3 *Statistical analysis*

6.2.3.1 *Microsatellites*

Presence of null alleles and scoring errors were assessed using Microchecker (V.2.2.3) (Van Oosterhout et al. 2004); each locus was tested to determine whether or not it was exhibiting Hardy-Weinberg Equilibrium (HWE). GENEPOP (V.4.2) (Raymond & Rousset 1995) was utilised to perform HWE tests with a presumed excess of heterozygosity, and with a deficiency of heterozygosity as alternative hypotheses explaining the lack of HWE. GENEPOP was also used to estimate the frequency of null alleles that could explain the lack of HWE. Additionally, GENEPOP was also used to test for linkage disequilibrium, through the use of Fisher's Exact Probability Test, as well as to test for the presence of private alleles. The remaining loci were analysed using Microsatellite Analyser (MSA) (V.4.05) (Dieringer & Schlotterer 2003) to determine observed and expected heterozygosities.

The number of sub-populations found within the 122 individuals sampled was determined using STRUCTURE (V.2.3.2) (Pritchard & Donnelly 2000). This programme was used to run 1.0×10^7 iterations, with a burn-in of 1.0×10^6. The number of populations (K) was estimated by simulating values of k between 1 and 15, each Markov chain Monte Carlo (MCMC) simulation being repeated three times. Results from these 45 MCMC simulations were collated using Structure Harvester (Earl 2012). Populations were subsequently mapped using ArcGIS (V.9.0).

The different populations were assessed to determine whether a genetic bottleneck had occurred using BOTTLENECK (V.1.2.02) (Cornuet & Luikart 1996). The populations were assessed using the Stepwise Mutation Model (SMM), as well as the Two Phase Model (TPM) using default parameters. By doing this, both types of mutation could be assessed. To determine if a population expansion had occurred, both *k* (Reich & Goldstein 1998) and *g* tests (Reich et al. 1999) were performed using the "Kgtests" Excel macro (Bilgin 2007). Further bottleneck analysis could have been carried out with

the use of Msvar (Girod et al. 2011), however this was not possible due to time constraints.

6.2.3.2 *Mitochondrial DNA(mtDNA)*

The mtDNA sequences were analysed as an entire population, as well as, by population, as assigned by STRUCTURE (see above Section 6.2.3.1). Analysed in DNAsp (V.5.10.01) (Librado & Rozas 2009) and Arlequin (V.3.5.2.2) (Excoffier & Lischer 2010), the nucleotide diversity (π), analyses of molecular variance (AMOVA), Tajima's D and mismatch distributions of the mtDNA sequences were determined. Analyses of molecular variance were used to assess the variance both among and within the populations. Mismatch distributions and Tajima's D were computed as these are informative about the populations' demographic history. Haplotype diversity was examined in NETWORK (V.4.613) (Bandelt et al. 1999) and a median-joining network was constructed. Haplotypes identified from the current study were plotted along with the additional haplotypes from Genbank (see above Section 6.2.3.1).

BEAST (V.1.8.2) (Drummond and Rambaut 2007) was used to create a temporally calibrated phylogenetic tree; mutation rates were estimated using time since divergence between *C. porosus* and *C. niloticus*, as given in Oaks (2011) and Genbank sequences of *C. niloticus* (JF502245.1) of the sequenced region. Three runs were conducted in BEAST, with 1.0×10^5 burn in steps and 1.0×10^6 steps of the MCMC algorithm. These results were combined using Log Combiner (V.1.8.2) prior to using Tree Annotator (V.1.8.2) to create a summary tree from the 30,000 phylogenetic simulations. Finally, TRACER (V.1.6.0) was utilised to construct Bayesian Skyline Plots (BSP) and lineages, analyses were carried out with a 10% burn-in (Rambaut & Drummond 2007).

6.3 Results

A total of 122 tissue samples were collected from both the Kinabatangan River (*n*=111) and a crocodile farm (*n*=11). All samples provided sufficient DNA of high quality for subsequent analysis.

6.3.1 *Genetic variation*

Microchecker indicated the potential presence of population structure when analysing the complete dataset simultaneously with a large proportion of loci seemingly presenting null alleles. Six of the microsatellite loci multiplexed failed to produce reliable results during fragment analysis across the 122 samples and were therefore removed from subsequent analyses. Forty samples were rerun to reinforce the impact of the remaining loci. Two additional loci, CpP302 and CUD68, were deemed to possess null alleles; this was established after suggested populations were subsequently assessed for excess zygosity. This resulted in far fewer loci exhibiting null alleles supporting the hypothesis that population structure was present. There was evidence of linkage disequilibrium in two loci, Cj131 and Cj18, but these were included in analyses as it was not found to be the case for all populations, or for the total dataset. There was no evidence of private alleles being present within the sample range. Overall microsatellite heterozygosity across the 10 loci was lower than expected, ranging from 0.22 and 0.85 (Table 6.3).

Table 6.3 Summary statistics table for each microsatellite locus, excluding those loci that displayed null alleles: 1) number of alleles 2) observed heterozygosity 3) expected heterozygosity.

Locus	A[1]	H_o[2]	H_e[3]
CpP1308	17	0.49	0.51
CpP309	19	0.74	0.87
CpP914	4	0.44	0.50
CpP208	18	0.77	0.84
CpP1001	4	0.22	0.41
CpP501	15	0.85	0.85
CpP311	21	0.77	0.89
CpP405	3	0.54	0.35
Cj18	9	0.68	0.73
Cj131	14	0.62	0.80

6.3.2 *Population Structure*

Three sub-populations were determined across the sample range; indicated by the large peak at K=3 (Fig. 6.4). Samples obtained from the crocodile farm were spread evenly throughout the populations indicating that individuals were likely sourced from the Kinabatangan River.

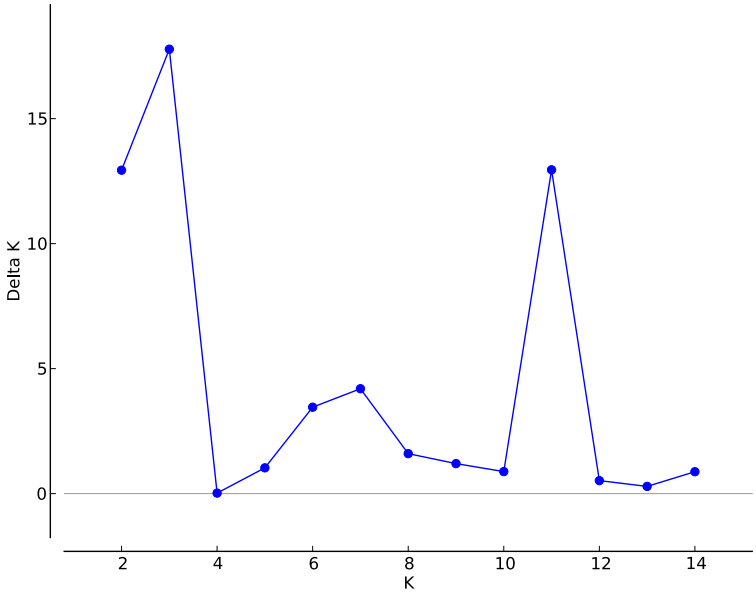


Figure 6.4. ΔK values against K indicating levels of population structure. Large peak at K=3 indicating that the Kinabatangan population is comprised of three distinct populations, where K equals the number of populations.

Individuals were sampled along a 200 km stretch of the Kinabatangan River; although a stretch of river without visible geographic boundaries, there remains the potential presence of human-mediated boundaries (see Chapter 3). Occurrence of populations appeared to be evenly distributed throughout the sampling range (Fig. 6.5) and suggests that spatial exclusion does not play a role in the maintenance of these three distinct populations.

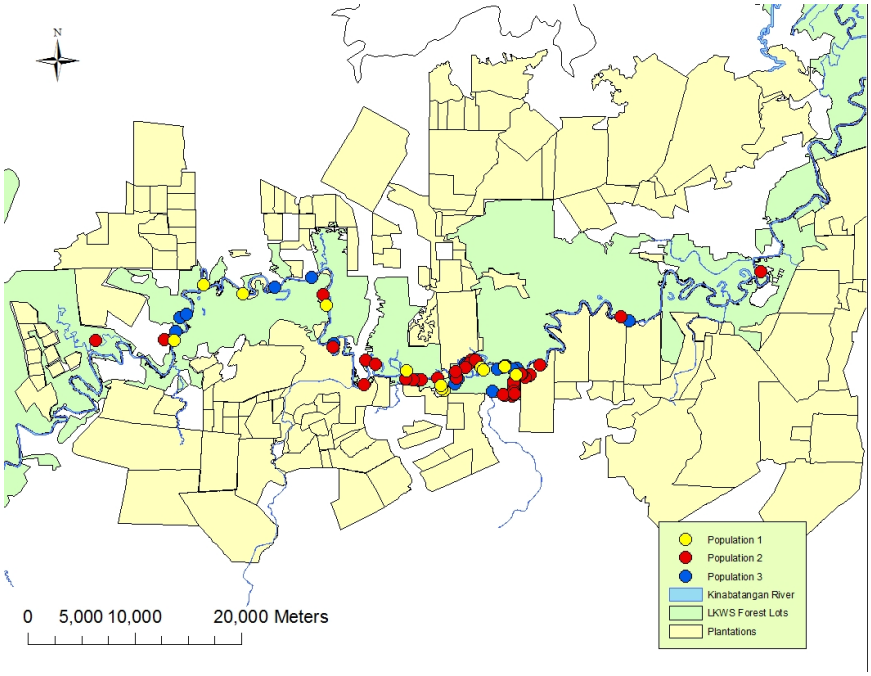


Figure. 6.5. Geographic distribution of the three populations as defined by STRUCTURE.

There appears to be little hybridisation between the populations, especially in Population 1 (Fig. 6.6). The presence of 11 farmed individuals of unknown capture locations were equally distributed throughout the three populations.

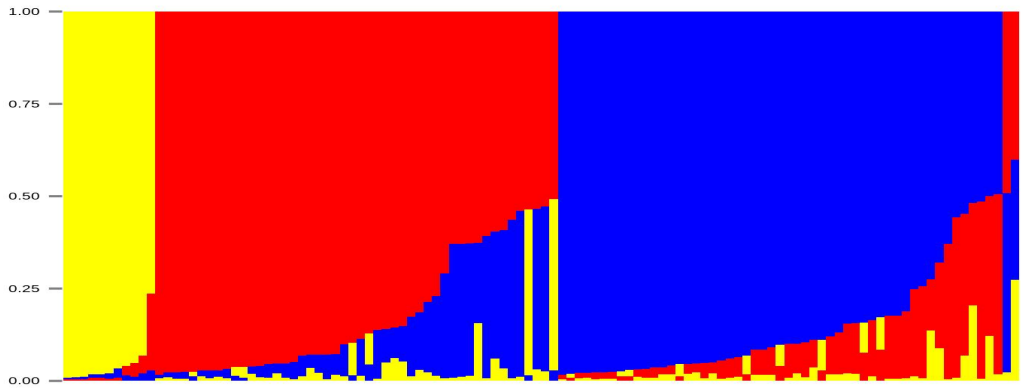


Figure 6.6. Clustering analyses indicating the mean population allocation of each individual. Plot shows low levels of hybridization between populations despite the lack of geographic separation. Yellow represents Population 1, red Population 2 and blue Population 3; several individuals are shown to exhibit true hybridisation between these clusters. Plot was created using STRUCTURE PLOT (Ramasamy et al. 2014).

Inbreeding coefficients (F_{IS}) were found to be not significant ($p = 0.15$) between populations, despite a high level of inbreeding found in Population 1 ($F_{IS} = 0.171$) and a very low level of inbreeding in Population 2 ($F_{IS} = 0.001$). Across the entire sampled population, inbreeding was relatively low throughout the Kinabatangan River ($F_{IS} = 0.063$). Population 1, with its high levels of inbreeding, was represented by fewer individuals than the other populations and, with a larger sample size, could have exhibited significantly higher levels of inbreeding than the other populations. Additionally, Population 1 also had the lowest levels of hybridisation with the other two populations (Fig. 6.6), which would be consistent with higher inbreeding levels.

6.3.3 *Mitochondrial DNA (mtDNA)*

MtDNA analysis resulted in the identification of four distinct haplotypes, with a low pairwise genetic distance of 0.8% and an average nucleotide diversity of 0.0019. These four haplotypes were coupled with the eight additional haplotypes taken from Genbank in a median-joining network (MJN) (Fig. 6.7). The MJN displays each of the haplotypes identified during the current study as unique (Fig. 6.7). There was a significant genetic differentiation between the populations ($F_{ST} = 0.183$, $p = 0.019$).

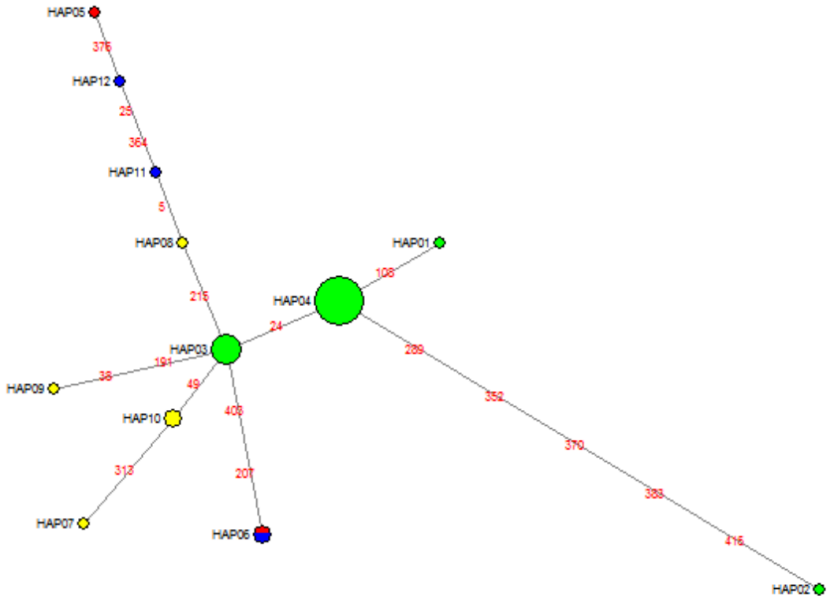


Figure 6.7 Median-joining network (MJN) haplotypes from different locations are denoted by differing colours: Kinabatangan (green), Southeast Asia (yellow), Queensland (Australia) (blue) and Northern Territory (Australia) (red). Haplotype 8 originates from western Kalimantan (Indonesian Borneo). Haplotype 6 is the only haplotype present in more than one location.

Analyses using BOTTLENECK suggested that neither the SMM (p = 0.403) nor the TPM (p = 0.090) detected a significant excess of heterozygosity through the use of two-tailed t-tests. This suggests a lack of evidence for a recent genetic bottleneck in any of the sub-populations, or for the population as a whole.

Mismatch distributions carried out in DNAsp suggest a population expansion with very few pairwise differences both in the entire sampled population (Fig. 6.8 a), and each of the sub-populations (Fig. 6.8 b-d). The green line indicated the expected number of pairwise differences under a demographic expansion, with the red line reflecting the observed frequencies. Observed frequencies closely reflect expected frequencies under the demographic expansion hypothesis. In addition, tests of Tajima's

D produced negative figures for each of the three populations (-0.8165, -1.31009, and -1.63982, respectively), and a mean negative Tajima's D value of -1.26, consistent with the suggestion of a population expansion in the past of these populations.

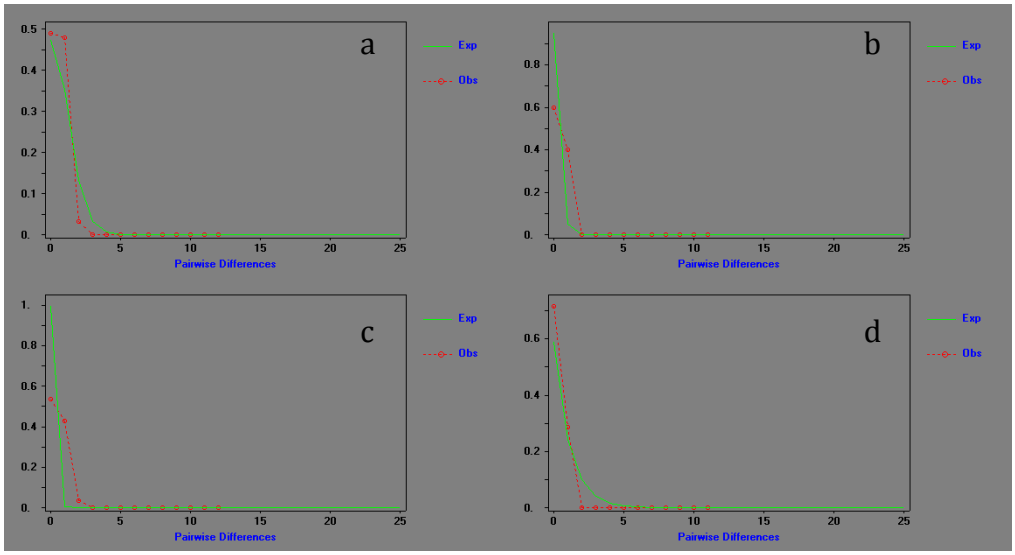


Figure 6.8 Mismatch distributions displaying the number of pairwise polymorphisms. Mismatch distributions across the entire sampled population (a), and for only Population 1(b), Population 2 (c) and Population 3 (d).

A Bayesian Skyline Plot (BSP) mapped and quantified the detected population expansion (Fig. 6.9). The BSP indicated a small three-fold population expansion that occurred somewhere around 25,000 years ago, however, the BSP had large confidence intervals.

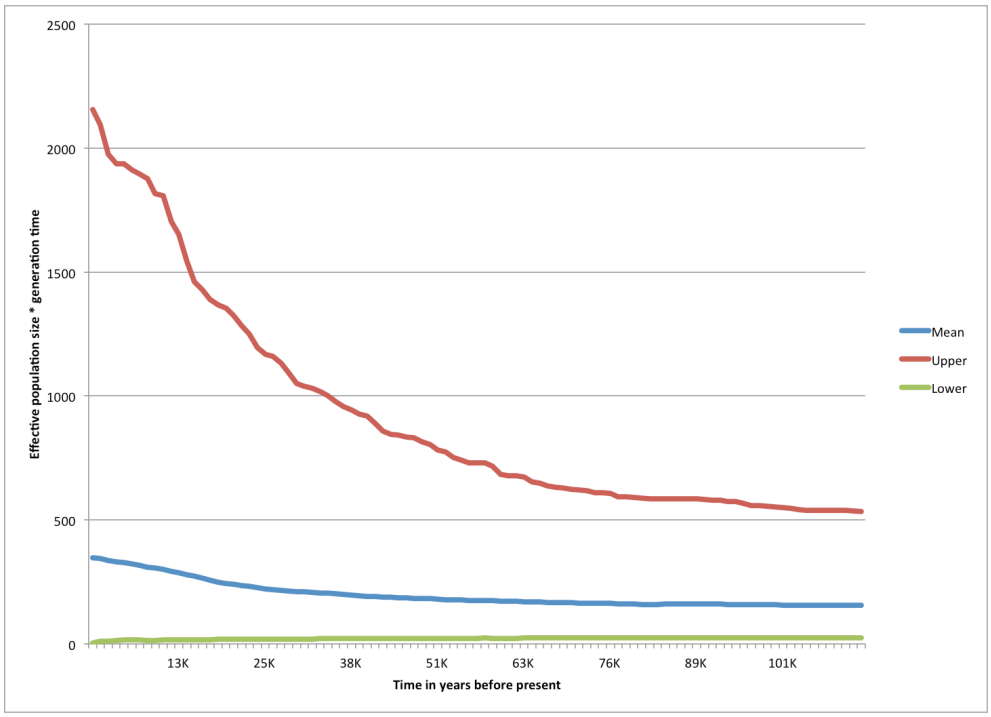


Figure 6.10. Bayesian Skyline Plot (BSP) showing the effective population size over the last 110k years. Note the small population expansion over the last 25k years. Green and red lines indicate confidence intervals, with the blue line showing the mean population expansion.

A neighbour-joining tree illustrates the low levels of divergence both between the individuals from the Kinabatangan, but also with many of the regional samples that were used from other studies (Fig. 6.10). The tree indicates that the majority of the Kinabatangan samples as well as individuals from Kalimantan, Southeast Asia, and three locations in Australia all form part of a single haplogroup. Individuals NT1 and Southeast Asia 3 appear to be highly genetically distinct from the reminder of the samples (Fig 6.10).

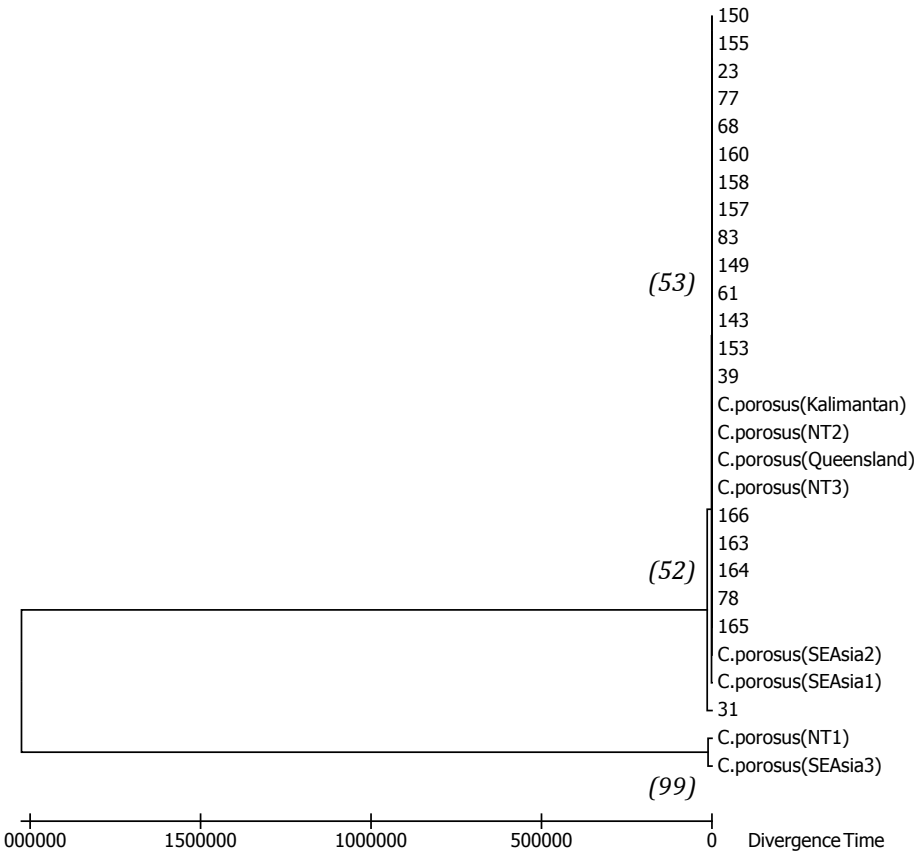


Figure 6.10 Evolutionary relationships between both the Kinabatangan samples and individuals sampled from across the region using the neighbour-joining construction method. Divergence times were based on mutation rate calculated from *C. niloticus* sequences given a known divergence time (Oaks 2011). Percentage confidence of each clade is denoted by bracketed figures.

6.4 *Discussion*

In many ways, estuarine crocodiles are unique, both among crocodilians and non-oceanic dwelling predators, in their ability to traverse oceans; they are rarely restricted by geographical barriers (Campbell et al. 2010). This has led to their expansive range and widespread distribution. As an ancient species, dating to approximately between 9 and 39 million years old (Oaks 2011), *C. porosus* has dealt with shifts in both climate and habitat suitability. The Bornean population of estuarine crocodiles has endured recent population fluctuations (Whitaker 1984) but this present study suggests that this has had limited impact on the population's genetic health. Whether

this is the result of more individuals than expected surviving the population decline, or that the decline was not over a sufficiently long enough period, in terms of number of generations, to cause a significant loss in heterozygosity, remains unclear. This is despite the fact that during the 1984 surveys, only 56 individuals were spotted over the course of 1,146 km of river surveyed (Whitaker 1984). This represented an average detection rate of just 0.05 individuals per km, far less than the 3.98 and 2.09 individual per km detected in two of Australia's major rivers during the same period.

The lack of a genetic bottleneck in the Kinabatangan population is consistent with the findings of other studies (Gratten 2004; Russello et al. 2007), and can probably be explained by the free movement of surviving (remaining) individuals, and the willingness and ability of transient females (see Chapter 3) to travel larger distances to find potential mates. This coupled with the brief nature of the population bottleneck (4-6 generations) ensured that high levels of heterozygosity were maintained. Such observations underline the importance of ensuring free passage for individuals up- and down-river, and raises questions regarding the consequences of the erection of non-intentional barriers, such as bridges (see Chapter 3), on the genetic health of the population should numbers fluctuate in the future. This is also an important consideration when individuals are culled (a management strategy under consideration in the area).

The finding of a population expansion in the Bornean population is corroborated by the findings of other *C. porosus* studies in other regions (Gratten 2004; Luck et al. 2012; Russello et al. 2007). Gratten (2004), for example, found an expansion dating back to the Pleistocene Epoch across maternal lineages. This author also described the entirety of the *C. porosus* population as belonging to just one evolutionary significant unit (ESU, Moritz 1994). The results of this present study suggest that the Sabah population also falls within this same ESU, with no significant differentiation from other sampled populations (Fig. 6.10). The population expansion found was small, with just a three-fold increase over the course of around 40,000

years. This could be attributable to changes in climate associated with the last Ice Age.

A relatively low level of mtDNA divergence was found (0.8%). This, however, was higher than both divergences found by Luck et al. (2012) and Russello et al. (2007). Such an overall low level of mtDNA genetic divergence is characteristic of individuals found throughout Southeast Asia (Luck et al. 2012) and appears to be characteristic across a range of crocodilians (Glenn et al. 2002; Ray et al. 2004; Schmitz et al. 2003). It has been suggested that Crocodylia exhibit such low levels of mtDNA divergence due to population bottlenecks during the 20th Century (Luck et al. 2012; Glenn et al. 2002). The lack of a bottleneck identified in the current study would, however, indicate that any genetic bottlenecks that did occur did not have a significant effect on the overall genetic health of the population. Any heterozygosity lost during such an event would take longer to be regained in the mitochondrial genome than the rapidly mutating microsatellites (Haavie et al. 2000). This could potentially be indicative of a bottleneck that occurred further in the past, at the species-level, from which the mitochondrial genome is still recovering.

The present study identified the presence of four distinct haplotypes, all of which were distinct from the eight, previously published, haplotypes used for comparison in this study. The new haplotypes were also distinct from the haplotype identified from East Kalimantan, Indonesian Borneo (Russello et al. 2007). This suggests a broader number of haplotypes could be present throughout Borneo.

The equal spatial distribution of individuals from each of the three identified populations indicate that the movement of females and transient males (see Chapter 3) are key in mate-selection and in ensuring that populations are retained. The equal inclusion of individuals from the crocodile farm located between the Kinabatangan and Labuk Rivers suggest that all specimens were collected from the Kinabatangan. Furthermore, with the collection

location of the individuals unknown and, potentially, from different positions along the river, this view supports the assumption that sampling of 111 individuals along a 200 km stretch of river was sufficient to provide a true representative of the overall population demographic. The lack of spatial segregation of sub-populations could possibly be a result of source-sink dynamics. Source-sink dynamics can produce non-spatially exclusive populations due to influxes of new individuals into an area during times of severe population depletion (Ferreira da Silva et al. 2014).

There are isolated reports of the existence of a Bornean crocodile, *C. rainus* (Ross 1990). These claims, however, are based solely on morphology of museum specimens and lack credibility, with suggestions that *C. rainus* specimens are in fact the Philippine crocodile (*C. novaguineae*) (Gratten 2004). The present study found no evidence of anything approaching species-level divergence between any of the 122 individuals sampled, suggesting that only one species is present in the Kinabatangan River. Reports (Stuebing et al. 2006) record the historic presence of the tomistoma (*Tomistoma schlegelii*) in the Kinabatangan, however there was no morphometric or genetic evidence found in the present study of any surviving population.

Future work should focus on establishing the genetic relationships between populations from different rivers in Sabah; this would not only help provide an increased understanding of the health of the state's crocodiles. This would also allow for estimates of inter-river migration and the establishment of whether individual rivers harbour "at risk" populations following population bottlenecks. An increased understanding of the implications of different management strategies must, however, be understood before those strategies are implemented. This is also true for any future plans for crocodile culls. Whilst evidence from this present study suggests that a carefully managed cull would have few detrimental effects on the genetic stability of the population, this is not necessarily the case for all populations in Sabah.

The identification of non-spatially exclusive populations reinforces the viewpoint that barrier erection should be avoided along the Kinabatangan River. The presence of a large bridge (see Chapter 3) has the potential to interrupt gene flow between individuals. For crocodiles, these bridges represent non-conventional habitat fragmentation. Proposed future bridges would intensify this issue, not just for crocodiles but also for many terrestrial species such as elephants, felids and primates.

Chapter 7 General Discussion and Conclusions

The Kinabatangan River basin has witnessed a large increase in crocodile numbers over the past 30 years, increasing from 0.21 km^{-2} in 1984 (Whitaker 1984) to 0.53 km^{-2} in 2011 (Luke Evans, *unpubl. data*). Increases in population numbers have resulted in an increase in human-crocodile attack figures. Potential shifts in the distribution of prey, and thus its availability, as a consequence of habitat fragmentation mainly attributable to oil palm conversion, are likely playing a key role in these increased levels of conflict. The highly fragmented landscape found in the Lower Kinabatangan Wildlife Sanctuary (LKWS) makes it an important model for studying lowland tropical rainforest ecosystems; these are areas that are globally most at risk from conversion, but particularly so, throughout South East Asia (Achard et al. 2002).

An understanding of population structure and behavioural relationships are important steps in mitigating conflict, and ensuring that crocodilians and humans can co-exist. Developing such a knowledge-base is an essential part of creating an effective management plan for the region's crocodiles; this provided an important focal area and objective for the current study. In doing so, the study additionally provides an insight into the ecology of the estuarine crocodile that has not previously been examined. As well as corroborating some recent spatial findings on the distribution and dispersal of estuarine crocodiles in Australia (Campbell et al. 2013), the study also provided an opportunity for numerous novel explorations. The genetic work on the crocodiles of the LKWS (Chapter 6), for example, provided an insight into both the genetic effects of the population bottleneck that occurred during the 20th Century, as well as commenting on the history of the population over recent millennia. The genetic resilience of a population that is seriously depleted has important implications for management of population recovery for a whole range of organisms.

The experimental chapters in this thesis sought to test a number of hypotheses. Chapter 3 considered the levels of territoriality shown by the estuarine crocodile and attempted to determine whether individuals of either sex were holding permanent home ranges. The determination of the presence of both territorial and nomadic males is consistent with the findings of Campbell et al. (2013). Females were found to observe similar ranging patterns, however due to the small sample size further work to confirm this is required. Territory sizes were much smaller than those reported in previous studies on Australian crocodiles (Campbell et al. 2013); this is probably attributable to increased prey availability in the LKWS locale permitting a higher carrying capacity for the habitat, with subsequently more individuals housed per km^2. By assessing temporal movements of individuals it was possible to ascertain crepuscular peaks in movement rates at both dawn and dusk. The information gathered on movement rates and patterns, along with a better understanding of territoriality, has important management and conflict avoidance implications. For example, the findings from this project could be used when disseminating information and advice to highlight times and areas of high crocodile attack risk to local people.

The use of Unmanned Aerial Vehicles (UAVs), or drones, is tending to become commonplace within conservation biology, as indeed in many other scientific disciplines. This is mainly the result of developing technologies proving cheaper and studies becoming more repeatable. The successful identification of crocodile nests using drones (Chapter 4) is a novel use and has enabled the first identification of a crocodile nest in the LKWS. The ability to identify nests using drone flights enabled a first attempt at assessing their spatial distribution (Chapter 5). By detecting a total of five nests, through surveying a relatively small area of habitat, nesting appears, as had been hypothesised, to be predictable; such a finding can be used to refine, and make more efficient, future nesting surveys. Using “fuzzy overlay” functions as a predictive modelling approach to nest location and distribution is an obvious way forward in this venture. Nesting was found to

occur in medium-high levels of disturbance. This has major conservation and management implications as it suggests that crocodiles are very likely to be able to persevere in highly fragmented habitats, with nests in oil palm even possible. The location of nests in close proximity to human activity must be considered when writing management plans and also when considering what the carrying capacity of a river might be.

Chapter 6 dealt with a study exploring the population genetics of the estuarine crocodiles of the LKWS. In particular, the study examined both how individuals were segregated within the river and how the genetic health of the population had been affected by population instability potentially derived from human hunting activities on crocodiles. The findings that the crocodile population had not undergone a recent bottleneck proved the resilience potential of even relatively small populations, as well as their ability to rebound. The existence of three geographically aggregated populations, or haplogroups, provided strong evidence that individuals are able to move freely throughout the river. This supports the hypothesis that females are largely transient and will travel in order to mate preferentially (Chapter 3). Medium-high levels of inbreeding found within the rarest of the three haplogroups did, however, provide cause for concern should, for example, barriers such as bridges prevent females from reaching desired mates in the future. The historical population genetics examined in this project suggests that a population expansion did occur within the LKWS population some 25,000 years ago. This could have been as a consequence of changes in climate arising during the last Ice Age (26,000 – 13,000 years before present) (Richmond & Fullerton 1986).

These key findings provide useful signposts for future management strategies, not only in terms of human safety, but also with regard to estuarine crocodile population stability. Males provide the majority of human conflict concerns and are thought most likely to be responsible for the majority of injuries and fatalities (Caldicott et al. 2005). Females can, however, become increasingly aggressive during the nesting season; this

elevates nesting in areas within, and directly around, oil palm plantations to a level of major safety concern. The identification of important nesting areas, containing characteristic habitat features common to all identified nests (Chapter 4 and 5), ensures that people can be informed that areas containing these traits are treated as "high risk" locations and are avoided.

To maintain a level of stasis between human and crocodilian populations, the overall health of crocodilian populations is of fundamental relevance. Assessment of the consequences of anthropogenic activities on crocodilian welfare must, therefore, become a priority research activity. The identification of human-erected barriers (Chapter 3), with their associated consequences in terms of crocodile movement and distribution, has important management considerations and provides further emphasis to the notion that animal ranges must be considered when "essential" habitat fragmentation must occur. The genetic implications of such barrier formation are yet to be felt (Chapter 6); they will have important, and probably far-reaching, inbreeding implications over future generations. The identification of non-geographically isolated haplogroups or populations reinforces the importance of free movement throughout the habitat.

7.1 Management

The management of crocodilian populations has seen varying levels of success over the last 40-50 years. Populations in more developed countries, such as Australia and the United States, have seen the establishment of highly successful management strategies along with, in some cases, harvesting schemes with up to and including tens of thousands of adult crocodiles being removed from the ecosystem annually (Moyle 2013; Saalfeld & Fukuda 2013). In general, attack numbers in these countries tend to be lower than in developing nations, however, the species that are present in any particular geographical region does have a role to play in determining attack numbers (Caldicott et al. 2005; Langley 2005). In some developing nations where carefully identified management plans have not

been implemented, a rise in crocodilian numbers has resulted in spiralling attack numbers and revenge killings.

Ecotourism is a burgeoning market and has led to general widespread interest in conservation issues from the public (Stronza 2007). This is true of the crocodile and despite its dangerous nature, the increasing popularity of "jumping crocodile" tours in Northern Australia has resulted in a conservation success story (Ryan & Harvey 2000) and the generation of at least $2 million AUD (~£1 million) of annual income (Tisdell et al. 2004). As of 2001, tourism receipts across the state of Sabah were estimated to total RM900 million (Ringgit Malaysia) (~£142 million); approximately 60% of this revenue (RM540 million) (~£85 million) was assessed to have been sourced from ecotourism expenditure (Sabah Wildlife Department 2010A). Large crocodilians can be found throughout the LKWS, as well as the rest of Sabah, and their presence contributes an estimated RM27 million (~£4.2 million) to the annual ecotourism industry (Sabah Wildlife Department 2010A). This highlights the economic value of sustained or growing crocodilian populations. As noted throughout this thesis, the Kinabatangan River harbours a thriving population of estuarine crocodiles that has recovered rapidly following significant declines during the 20th Century. The specific study site, directly down-river from Batu Puteh village, was selected by the government as an ideal location for crocodile-based ecotourism in response to the high numbers of large individuals readily visible by boat in the region (Sabah Wildlife Department 1997). The scheme, however, was never fully implemented due to a lack of funding. The potential of ecotourism to provide a financial incentive to crocodile conservation and management should not be underestimated; it also has the potential to change negative opinions towards this important apex predator.

In terms of future management strategies, areas identified as having high levels of territorial male activity during this project (Chapter 3) were deemed as being areas of higher risk of human attack. In an attempt to reduce likely escalation of human-crocodile conflict, signs (Figure 7.1) are

being placed at strategic locations throughout the LKWS to provide ample warning to local people operating in those areas.

Based on the findings of this current study specific management strategies for the LKWS should include protection of the remaining habitat in an attempt to ensure that high levels of prey remain available for the expanding crocodile population. The erection of bridges should be carefully considered so that individual crocodiles do not become isolated, leaving them unable to access much of their prey. Prey defence (often against fishermen) and hunger could be a major factor in future human-crocodile conflict. To prevent, or at least lessen, conflict with female crocodiles, areas that meet all requirements for nesting (as determined in Chapter 5) should be protected, ensuring that egg-laying female crocodiles are not forced to nest within oil palm plantations. If these recommendations are not taken under advisement, a major concern will be that the only tangible recourse will be the culling of the LKWS population. This will hamper any efforts to construct an ecotourism industry around centred on what are some of the largest crocodilians in the world.

Figure 7.1 Sign board advising people that the area has high levels of crocodile activity and not to enter the water.

7.2 Future Research

This study was restricted to one, albeit the largest, of Sabah's rivers. Future research should undoubtedly investigate the state on a more widespread scale, especially in areas surrounding rivers where oil palm completely dominates the landscape. Such an exercise would allow wide-scale population estimates to be made; this could provide the data evidence needed to downgrade the conservation status of the estuarine crocodile in accordance with CITES. To do this would require demonstration of "non-detriment findings", essentially the displaying of a stable, or increasing, widespread population. Downgrading the species would provide greater avenues for increased revenue from the crocodile meat and skin industries; it would also ensure that local people place a value on the presence of crocodiles. As previously discussed, management and large predator conservation is far more effective when there are financial advantages to be had by their thriving in an ecosystem.

In terms of building on specific findings from this study, a number of areas of limited knowledge and understanding remain. For example, further examination of the effect of human-constructed barriers, such as the bridge identified in Chapter 3, on crocodilian movements, is essential as continued worldwide fragmentation of ecosystems catalyses a spiralling number and density of such barriers. Verifying the extent of the effects of such structures requires prioritisation, as indeed does the exploration of whether any other form of human disturbance could produce such barrier effects. Expansion of nesting surveys using drones is a virtual necessity, and certainty, as rapid technological advances and reductions in cost will ensure that single flights can cover far greater areas of potential nesting habitat (Chapters 4 and 5). This would enable detailed habitat utilisation maps to be produced allowing the protection of "key" areas for crocodilian nesting. In terms of population genetics, as previously mentioned, state-wide surveys are already planned (Chapter 6); these will allow detection of not only inter-river travel but assessments of genetic health of smaller populations, in particular those that are even more adversely affected than the LKWS. In these rivers, numbers of

individuals is likely to have fallen far lower than in the LKWS, these populations may have been more adversely affected by previous population bottlenecks.

7.3 Project limitations

Working on the largest extant reptile represents a huge challenge and ensuring that adequate training experiences, and appropriate, standardised and sufficient data were obtained over a limited period of four years had its challenges! Through trial- and-error-based trapping and baiting approaches and attempts, an efficient capture technique was achieved, but only by Year 3 of the project. This clearly limited the total number of crocodiles captured. Regardless of manufacturers' descriptors, satellite tags, as with many new technologies, were not always reliable and despite the use of three different tag systems a truly successful setup was found for use in the LKWS. Unfortunately, the limited sample size of seven tagged individuals does mean that the findings, while accurate and convincing, must be tempered with a degree of caution. Note should also be made that, along with the trapping challenge, cost limitations on satellite units can be restrictive.

The use of drones proved an exciting and alternative means of assessing nest distributions. Being reliant of developing technology does, however, bring with it an associated "high-risk", particularly when usage is of a novel kind. It was not, however, the technology itself that provided the greatest challenge, but the environmental and climatic conditions during the sampling and surveying period. Complete submergence of the area following heavy precipitation led to the majority of potential nests detected not being visible and available for study.

A project of this nature, were it to be carried out in the future would ideally need more than the three field seasons that were feasible during this project. Only by doing this can a sufficiently large enough data set be collated in what are very difficult field conditions.

7.4 Conclusions

The study finishes with a plea! The completion of any future work of this nature is gravely threatened by the difficulties experienced in acquiring funding for research providing a better biological, ecological, economic and survival understanding of these most ancient of predators. Crocodiles play a crucial role in ecosystems throughout the world and their perseverance is key in trophic dynamics and has potential pharmaceutical importance. Human-crocodile conflict mitigation relies on education of local people and maintenance of habitat to ensure a suitable prey base. Understanding both in terms of wild and captive populations is fundamental to a harmonious relationship between both 'man and beast'.

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